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PREDISPOSITIONS OF CONSCIOUS PERCEPTION: FROM CORRELATION TO CAUSATION

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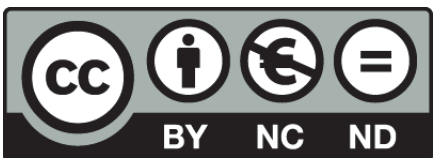

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Overview

Human mental life is accompanied by oscillatory signals that send information across distributed neural networks. Whether a stimulus reaches or escapes our conscious experience is influenced by the state of the brain in that moment, reflected in cerebral electrophysiology. Our understanding of this brain activity has grown vastly in recent years, thanks to leading advances in electro- and magneto-encephalography (EEG and MEG, or M-EEG, which enable us to monitor the electric brain signal) and recent developments allowing the direct modulation of endogenous oscillatory components that underlie cortical functions. Transcranial current stimulation, particularly the variant with alternating current (tACS), putatively lets us assess and gauge the role of oscillations on cognition. Several studies have confirmed that tACS can influence neural mechanisms and behavior, even conscious access. Until recently, cerebral activity during stimulation could not be assessed and observations were limited to the aftereffects.

The aim of the project described in this dissertation is to investigate the validity of a pioneering procedure that can recover brain signal during simultaneous MEG and tACS. Then, exploiting this approach, we furthered our grasp of how the neural system is altered by transcranial stimulation and the complex relationship between the external current and the internal mechanisms of the brain. The overall goal is to explore our ability to manipulate neural signatures in ongoing activity and the conscious perception of an upcoming stimulus.

Chapter 1 provides the reader with a general introduction of current studies and theories behind tACS influence on cognition and behavior. After a description of what tACS is and what it does, the focus is mostly on cutting-edge methods combining tACS and M-EEG, network connectivity and graph theoretical frameworks to study cognitive processes. At the end of the introductory chapter, we indulge on applications and consequences of these approaches, as well as open questions about our understanding of the prerequisites of conscious perception that drove the experiments described in the following chapters.

Chapter 2 reports the first study in which we addressed the feasibility of concurrent tACS and MEG, the prerequisite for the rest of the project. *Chapter 3* and *4* present studies that better delineated what happens in the brain in terms of oscillatory phase, connectivity and the dependency of tACS effects with the ongoing brain state during electrical stimulation. We addressed some key issues on the mechanisms of action of tACS and its sensitivity to in-vivo brain networks.

Chapter 5 provides preliminary results of a study employing a near-threshold task paired with tACS and MEG in the context of conscious perception. We stimulated prestimulus brain rhythms in sensory cortices to see if their strength and connectedness with the rest of the brain could determine whether a stimulus will be perceived or not.

Chapter 6, after a recapitulation of the main results in a broader perspective, discusses the meaning and the limitations of the experimental findings and how these extend our current knowledge.

Chapter 1. **Introduction and Background**

1.1 **The Importance of Oscillatory Brain Activity**

Cognitive operations and cortical functions are mediated in the brain by local and long-distance networks of neurons (Buzsáki, 2006). Neural activity reflects the functional organization of this system and can be detected by means of non-invasive electrophysiological techniques, such as electro- and magneto-encephalography (EEG and MEG, or M-EEG), which record with high temporal resolution the synchronized mass signal of large groups of firing neurons. Network dynamics of various scales interact to generate periodic fluctuations in the action potentials of neuronal populations, giving rise to synchronized activity all over the brain. Recorded neural activity exhibits patterns in the frequency spectrum, and the correlations of these oscillatory characteristics with processes of cognition and behavior are a fundamental matter of study of neuroscience. These so-called *brain oscillations* reflect the balanced system reacting to external and internal stimuli beneath the functional aspects of cortical operations. Spontaneous and endogenous rhythmic activity has several frequency components that are specific to cerebral regions, states and functions. They predict performance in a considerable range of perceptual and cognitive tasks (Wang, 2010) and show alterations in specific neurological and psychiatric conditions (Weisz et al., 2005; Schnitzler & Gross, 2005; Uhlhaas et al., 2008, 2012).

For example, one of these brain rhythms, the alpha frequency (about 10 Hz), observed in the raw signal since the beginning of human electrophysiological recordings (Berger, 1929), is theorized to reflect network-driven local excitability with functional importance for stimulus detection (Jensen & Mazaheri, 2010). This has been observed with M-EEG, correlating visual detection performance both to alpha power amplitude (e.g., Thut et al., 2006) and to phase (e.g., Busch et al., 2009). Additionally, it has been shown that oscillatory alpha activity prior to sensory stimulation predicts conscious access (Hanslmayr et al., 2007; Romei et al., 2008; van Dijk et al., 2008; Gallotto et al., 2017). For instance, visual stimuli that are presented at low contrast will be seen depending on the ongoing posterior alpha power (Lange et al., 2013). The importance of alpha phase for conscious access has also been shown by Neuling et al. (2012a), who found that the phase of an applied oscillating current stimulation at alpha frequency determined auditory detection thresholds. This finding confirms the importance of brain rhythms and provides a causal link between alpha phase and conscious perception.

1.2 **From Correlation to Causation to Brain Signal**

Over the years, recordings of M-EEG have shown that brain rhythms reflect coding and transfer of information of external and internal states by correlating behavior with the amplitude and the phase of their frequencies (Cohen, 2011). However, with methods that manipulate physiological frequencies directly (as in the study mentioned above, Neuling et al., 2012a), we can now determine whether oscillatory activity actually plays a role in cognition and behavior. Neurostimulation is initiating a paradigmatic shift from correlation to causation in neuroscience, in particular for studying the function of brain oscillatory activity and cortical connections. By altering neural activity directly, as an independent variable, we can follow resulting changes in brain signal and in performance. These methods include non-invasive brain stimulation, like transcranial magnetic and electric stimulation (TMS and tES, or tCS). With a version of tCS using alternating current (transcranial alternating current stimulation, tACS), the neurostimulation can be set to a specific frequency of interest. Until recently, the only dependent variable in paradigms using non-invasive brain stimulation was usually behavior. We could establish causal effects of neurostimulation on behavior, but not on brain activity during stimulation itself.

Physiological recordings and targeted modulation of ongoing neuronal oscillatory activity have been two separate lines of research, one observing non-causal neural correlation with behavior and the other assessing causal relevance without looking at the impact of neurostimulation on brain physiology. Even though interesting behavioral and electrophysiological aftereffects of transcranial stimulation have been observed (Herrmann et al., 2013), what contributes to them is for the most part undetermined. Possibly thanks to short-term plasticity, tACS results in post-stimulation changes in amplitude, phase and coherence of resting brain activity (Hermann et al., 2013; Battleday et al., 2014). This modulation has also bearing on complex cognition and behavior, but a complete validation of offline aftereffects can only be achieved by understanding how they are created online (Vossen et al., 2014; Schmidt et al., 2014). Although these outcomes are seen consistently, their reliability is low and a big part of the impact on brain electrical signal and cognitive process is unknown. The nonlinearity of tACS effects (Roberts & Robinson, 2012; Reato et al., 2013) and their state-dependency (Neuling et al., 2013) expose the inadequacy of current *causality* assumptions. Unfortunately, what happens in the brain during ongoing electrical stimulation is difficult to identify due to the massive artifact, which is inextricable from the recording (especially at the stimulated frequency).

However, emerging techniques can now resolve brain signal even while transcranial stimulation is being applied (*Fig. 1.1*). While concurrent TMS and EEG studies can now be considered common, only a few studies using simultaneous tCS and M-EEG exist. The EEG samples during and in the first few milliseconds after the

massive TMS burst are all the same unrecoverable, yet because the artifact is consistent and non-saturating with tCS we can now monitor oscillatory activity even during neurostimulation. Given the significance of cerebral oscillatory signal for normal and altered cognition and behavior, tACS, suitable for stimulating a particular brain rhythm, can further our comprehension of brain physiology and function when coupled with M-EEG. It can also address how network-driven oscillatory mechanisms shape broad cognitive abilities. A clear understanding of how these techniques affect the brain in real-time would not only underpin the causal role of brain oscillations, but also provide a foundation to restore synchrony and treat atypical brain oscillatory activity and, possibly, enhance normal cognition (Giordano et al., 2017).

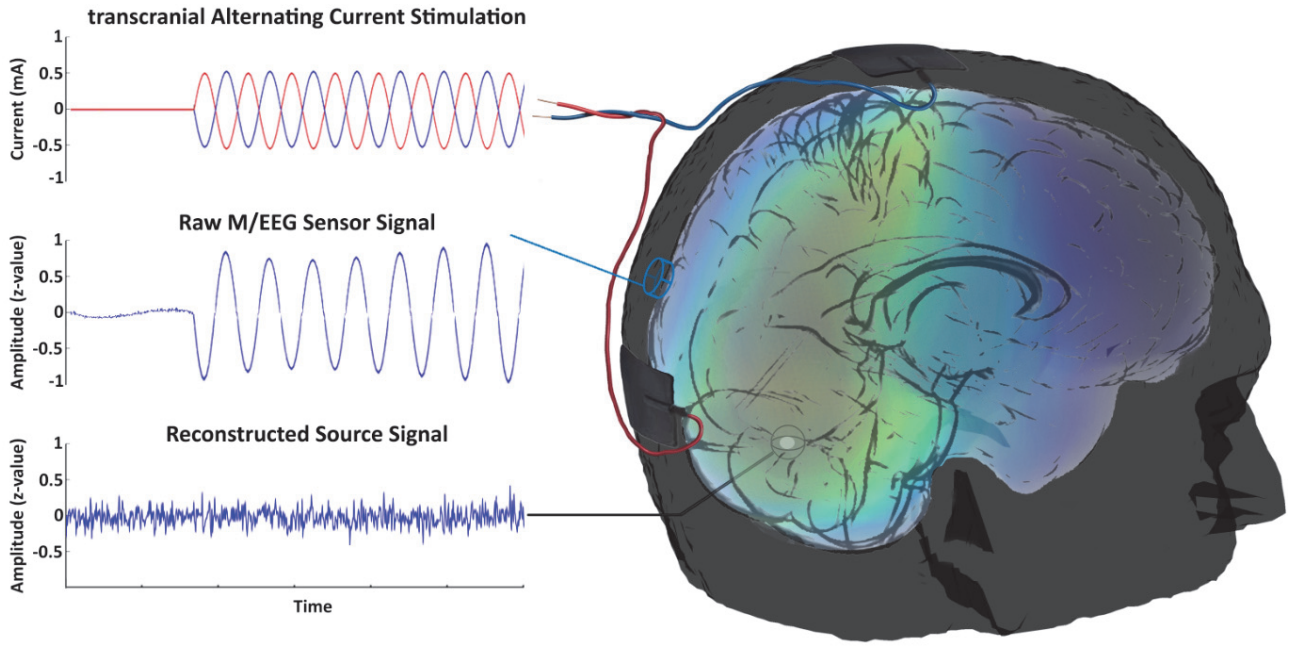


Figure 1.1 ***Recovery of Brain Activity under tACS***
A typical tACS and M-EEG setup and the signals involved. Cerebral electrophysiological signal can be extracted despite the massive stimulation artifact present at sensor level recordings. The colored brain shows the localization of power increase of the frequency of stimulation for a Cz-Oz tACS montage.

We refer to several reviews illustrating tACS more in-depth in its aspects regarding physiology (Reato et al., 2013), plasticity (Herrmann et al., 2013), its theorized effects on neural networks (Ali et al., 2013; Battleday et al., 2014), research (Antal & Paulus, 2013; Brignani et al., 2013; Fröhlich et al., 2014), clinical applications (Brittain et al., 2013; Kuo et al., 2014; Parkin et al., 2015) and neural correlates of conscious perception (de Graaf & Sack, 2014). In this introductory part of the dissertation, we try to disambiguate notions of this method slanting across these different fields, putting them together with the purpose of drawing a constructive perspective. We therefore illustrate what tACS is, its mechanism and limitations. Coming back to the recent

advances allowing the recording of the neural signal during tACS, together with their criticism and implications, we then propose that combined tACS and M-EEG could be integrated also with connectivity measures and become a potent tool to study cognition. We argue that only by reconciling the interaction between tACS and recorded endogenous oscillations in a framework that takes into account also the long-range network level, we will be able to understand the instantaneous effects of transcranial stimulation and, subsequently, how the observed state-dependent aftereffects are generated. Then the chapter moves toward the aim of the project and introduces how tACS can be used as a tool for unraveling the neural basis of the predispositions to conscious perception.

1.3 **Current Non-Invasive Brain Stimulation**

Transcranial current stimulation can modulate the intra-neuronal local field potentials and, consequently, the endogenous excitability of the cerebral cortex. Electrical stimulation of the brain has been attempted since we could master electricity, but this dated method is regaining popularity thanks to recent *in vitro* and *in vivo* recordings and modeling. Many relatively recent electrophysiological studies increased our understanding of how weak current affects neuronal networks' dynamics (Fröhlich & McCormick, 2010; Ozen et al., 2010; Reato et al., 2010). The blooming attractiveness of transcranial stimulation also follows preliminary yet encouraging results from treatment of neuropsychiatric disorders (Brittain et al., 2013; Kuo & Nitsche, 2012) and non-clinical applications for cognitive enhancement (Antal & Paulus, 2013; Giordano et al., 2017).

In research, the old acronym tES is still in use, which comes from the longstanding term *transcranial electrical stimulation*. This classification also refers to electro-convulsive therapy (ECT, the electroshock technique popular in the 70s used to treat grave psychiatric disorders, which is still used for conditions refractory to typical pharmacological treatment) and obsolete strong square-wave electrical stimulation able to evoke motor potentials. Consequently, some research groups are starting to use a different expression and abbreviation to distinguish the two techniques, tCS as in *transcranial current stimulation*. Contrary to ECT and motor-evoking stimulation, tCS is administered at a very low intensity, shows no major side effects and it can be imperceptible. The electrical stimulation is applied via two or more electrodes, in target locations relevant to the experiment (e.g., over Cz and Oz to stimulate occipital/parietal areas, like in *Fig. 1.1*), encased in rubber sponges soaked with saline solution strapped to the head or attached to the scalp with electroconductive paste. Contrary to TMS, which triggers neuronal firing, tCS causes an increase in spontaneous activity (Stagg & Nitsche, 2011). For this reason, TMS, a more popular neurostimulation technique, is considered less safe (Brocke et al., 2005; Sparing & Mottaghy, 2008). TMS is spatially more precise than tCS, but it is also more expensive and relatively

harder to sham, to apply and to support on the head. TMS is very versatile with its many applications and protocols, but tCS has possibly even more, with a wide range of choice in electrode number, size and placement, stimulation parameters, frequency, waveform, intensity, and duration. Ultimately, they have different effects and applications, and they can also be used at the same time (e.g. testing with TMS excitability changes prompted with tCS in the occipital or motor cortex; Kanai et al., 2008, 2010).

There are many flavors of tCS, and more are branching out (for a review see Herrmann et al., 2013), but the two most common are transcranial direct current stimulation (tDCS) and the variant with alternating current (tACS). The difference between the two is the regime, that is the shape of the waveform of the electrical current used: continuous (with one direction of current flow) in the case of tDCS and sinusoidal (oscillating between positive and negative polarities) for tACS.

Neuronal firing rate increases under the tDCS cathode (the positive electrode) and decreases under anodal (negative) stimulation. As cathode and anode in tACS switch at a given rate, firing rate fluctuates according to the frequency of the stimulation (Reato et al., 2010). With tDCS it is possible to excite or inhibit the same areas by changing the direction of the current administered (anodic or cathodic). On the other hand, with tACS the stimulation can be confined to a specific frequency, which is also feasible but not ideal with repeated TMS (rTMS; Herrmann et al., 2013).

Theoretically, in tACS compared to tDCS, the oscillatory current is overall non-depolarizing (i.e. with a null net depolarization -and therefore safer according to Schmidt et al., 2014) and more reliable if using specific frequencies for the subject (like the individual alpha frequency, for example; Neuling et al., 2013). Firing rates respond proportionally to both direct and alternate current (even at very low intensities; Deans et al., 2007). The limited depolarization and the smaller drive on single neurons of tACS could also be the reason tDCS is more suitable to induce plastic changes (Minussi et al., 2012; Antal et al., 2008; Nitsche et al., 2008), but neuronal ensembles seem to be more sensible to alternate currents (Reato et al., 2013). tDCS is still more popular than tACS in clinical use (Zaghi et al., 2010; Kuo & Nitsche, 2012).

1.4 **Mechanisms of tACS**

Even though the intensity used in tACS and the resulting electric fields in the parenchyma are relatively low, this weak -yet persistent and synchronic- current induction has an observable impact. Individual neurons' firing rate is affected only limitedly, yet stimulation-induced oscillatory modulation emerges at the level of neuronal ensembles (Fröhlich & McCormick, 2010; Reato et al., 2010). Recent studies confirmed that tACS influences brain dynamics and behavioral tasks with frequency-specific effects (Ali et al., 2013). The

effectiveness of tACS correlates with its intensity, which is limited by safety regulations and the experimental design (Neuling et al., 2013; Helfrich et al., 2014b). The strongest and most consistently observed effects of tACS emerge when the applied frequency matches a fundamental one already present in endogenous activity (Fröhlich & McCormick, 2010); it seems that functionally relevant brain rhythms can be modulated more easily (see also: Thut et al., 2012). Stimulation also has a state-dependent behavior, and its efficacy and the duration of the aftereffects depend on the current brain state and task-related activation, even with the same stimulation parameters (Herrmann et al., 2013).

1.4.1 *Resonance and State-Dependency*

Oscillatory brain activity can be characterized by power and phase. Power refers to the amplitude of the oscillation at a given frequency band and it is an index of maintained cortical excitation. Phase is the point in the sinusoidal oscillation cycle, and reflects momentary excitability, prompting different processing cascades depending on the instant information arrives (Thorne et al., 2011; but see Zoefel & Heil, 2013). The nonlinear interaction of tACS with local neuronal ensembles concerns power in the phenomenon called *resonance* and the phase in *entrainment*. When the neural oscillators that generate network frequency signatures (Siegel et al., 2012) are immersed in alternate current, which is weak but fluctuating at their preferred frequency, then they *resonate*: their excitatory and inhibitory potential increases, resulting in higher power in that frequency band. If these gentle but properly-timed electrical perturbations are not fluctuating at the same rhythm of neuronal firing, then the oscillators *entrain* to the stimulation, aligning their phase and their frequency (or just the phase if the frequency matches perfectly, *Fig. 1.2*) to the tACS. However, it is not clear how and how much entrainment and resonance contribute to behavior and electrophysiology, even though these effects are consistent in modelling and *in vitro* studies, covered below.

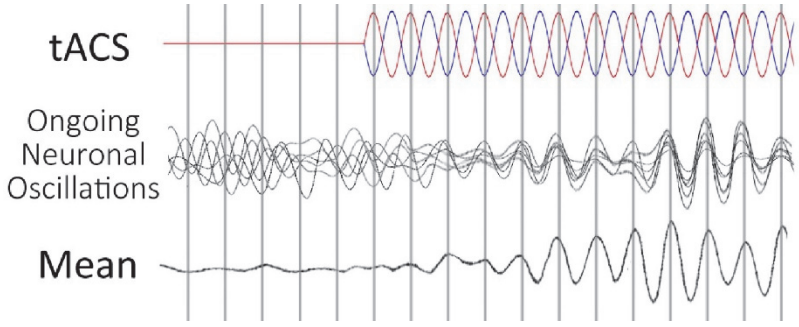


Figure 1.2 ***Entrainment***
This scheme illustrates the mechanism of entrainment in which phase-locked evoked activity shows an alignment of phases of ongoing oscillators (already oscillating at the stimulated frequency) to the tACS. If the oscillators' frequency is a few Hz off the the one stimulated, the entraining force can also alter their preferred frequency.

Neural network simulations, animal models and cortical slices show that depolarizing and hyperpolarizing electric fields enhance self-sustained local interactions of pyramidal cells and interneurons (Roberts & Robinson, 2012; Reato et al., 2013; Ali et al., 2013; Schmidt et al., 2014) as well as glia cells (Monai et al., 2016). Resonance at the same frequency of the stimulation is confined to low intensity and slow frequencies and, as they increases, the neural effects develop in a non-linear way (Reato et al., 2010; Roberts & Robinson, 2012). As mentioned above, frequencies that deviate excessively from the resting or task-activated oscillation do not drive or modify the network tendency to oscillate at its natural frequency. If the frequency is instead close to the endogenous one, the brain rhythm will align, and it is possible to manipulate directly oscillatory frequencies with behavioral consequences. For example, the individual alpha cycle correlates with the timing of crossmodal perceptual interaction in different subjects and by shifting alpha frequency with tACS entrainment, information parsing and integration will change. This again speaks for the causal role of alpha oscillatory activity in cyclically gating perception (Cecere et al., 2015).

Resonance in neuronal firing rates is seen concurrent to stimulation, but it produces analogous aftereffects post-tACS: an increase in the stimulated frequency's resting intensity. The alternating current might give rise to a persisting higher frequency power by inducing plasticity of coherent oscillators' synaptic weights (Hermann et al., 2013). If resonance is induced for some time, then this relatively short-term offline outcome can be observed, and thus aftereffects are state-dependent as well. In humans, tACS at the low frequency observed in non-REM sleep memory consolidation boosts this slow rhythm and improves retention, but only during sleep (Marshall et al., 2006). There are no alpha aftereffects after 20 minutes of alpha tACS with eyes closed, but alpha power is higher up to 30 minutes after stimulation with eyes open (Neuling et al., 2013). Kanai and colleagues (2008, 2010) similarly showed long-lasting changes after occipital stimulation in the dark for alpha but not for beta, and the reverse in well-lit conditions; they also showed a lack of these effects with frontal stimulation, proving valid localization -albeit coarse- in transcranial stimulation. The characteristics of resonance, dependent on brain regions and brain states, have been probed also with rTMS, with different networks showing specific endogenous frequencies driven by thalamic connections, with alpha in occipital, beta in parietal and high beta and low gamma in frontal regions (Rosanova et al., 2009).

1.4.2 *Entrainment and Cross-Frequency Modulation*

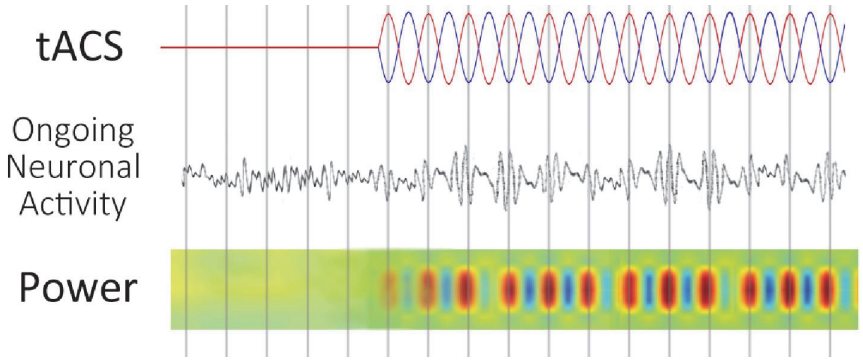
As for entrainment, depending on its definition, it may be achieved also by using rhythmic sensory stimulation (de Graaf et al., 2013; Henry et al., 2014; Spaak et al., 2014; Gray et al., 2015). However, it is yet unclear whether steady state evoked potentials, frequency tagging, optic driving or phase resets can be considered an entrainment of endogenous oscillations or they are simply stimulus-driven responses (Thut et al., 2011, 2012;

Keitel et al., 2014). In any case, behavioral entrainment is the most studied phasic modulation of tACS, and, unlike offline aftereffects, can be seen in concurrence with the stimulation. Performance follows the phase of tACS in visual tasks when entraining the endogenous posterior alpha rhythm (Neuling et al. 2012a; Helfrich et al., 2014a). Working memory is facilitated or impeded using theta stimulation (Polanía et al., 2012) and perception is altered using gamma (Strüber et al., 2014), showing opposite outcomes for in-phase or anti-phase fronto-parietal tACS. Again, these behavioral effects emerge only if tACS is applied at frequencies similar to the endogenous activity involved in the same task, revealed from previous EEG or MEG studies. Lucid dreaming is, for example, accompanied by prefrontal and temporal oscillations at 25Hz and 40Hz, and facilitated with tACS at only those frequencies (Voss et al., 2014). Similarly, frequency-specific tACS effects have been found in somatosensory, motor and visual systems (Kanai et al., 2008, 2010; Feurra et al., 2011).

Resonance and entrainment are not always treated as separate phenomena, as both are likely involved during oscillatory stimulation. Together they seem to be the result of more oscillators being engaged in functional networks with a specific rhythm (Hermann et al., 2013; Battleday et al., 2014). Aftereffects that do not necessarily regard phase are still made possible by entrained synchrony, that is network oscillations phase-couple and, at the same time, same-frequency oscillators' connections are strengthened plastically (but see: Vossen et al., 2014). Effects are often described as created by both resonance and entrainment in studies that are not inspecting them separately with multiunit recording and cortical slices.

Frequency bands different from the one used to stimulate can also show changes in tACS, as different frequencies are coupled in the brain: slow wave oscillations reflecting the excitatory and inhibitory equilibrium of the cortical network provoke amplitude fluctuations in higher frequencies (Buzsáki, 2006). Slow wave tACS can influence induced rhythms that are faster than the ones stimulated, with the power of high frequency oscillations following the crests and the troughs of the tACS cycle (*Fig. 1.3*). This usually entails brain rhythms in the gamma band modulated by frequencies below low beta (< 15 Hz, like alpha, theta and delta; Reato et al., 2013). This cross-frequency modulation is distinct from same-frequency resonance and entrainment and it is sometimes dubbed just with *modulation*. Unless they are being specifically investigated, the terms resonance, entrainment and modulation are often used interchangeably in studies describing tACS results.

Figure 1.3 ***Modulation***
Induced activity (power / amplitude) of frequencies higher than the one stimulated can be influenced by the cycle of the stimulation, showing cross-frequency coupling. Slow brain rhythms, like theta and alpha, are usually the driving frequencies influencing faster ones, like gamma.



1.4.3 *Effectiveness in the Non-Invasive Case*

To recapitulate, from invasive studies on animal models we know that tACS can manipulate temporal rhythms of excitation-inhibition in neuronal firing. This results in entrainment, an alignment of the phase of endogenous neural oscillators in the network, followed by resonance, an increase in power of the frequency matching the one of the stimulation (Fröhlich & McCormick, 2010). Entrainment also describes the shift of the peak frequency of natural neuronal firing to match the external drive (Reato et al., 2013). These effects are also dependent on the state of the cortex that the stimulation reaches (Silvanto et al., 2008; Neuling et al., 2013).

However, this might not be the mechanisms of action in the non-invasive case. Most of the current injected in the transcranial application dissipates throughout the scalp and the skull, while only an electric field and a current density of one order of magnitude smaller arrives in the brain (Miranda et al., 2013). In most non-invasive human studies with a 2 mA safety limit (Minussi et al., 2012; Nitsche et al., 2008), tACS does not seem to influence neuronal firing directly and efficiently. This has been ascertained for some years (Miranda et al., 2006) and this problem is frequently brought back, creating alarmism on the efficacy of tACS (Underwood, 2016) and shedding doubts on the numerous results already obtained with this technique. Behavior and brain signal could still be influenced by visual- or somato-sensory entrainment, from sub-threshold retinal phosphenes or cranial nerves stimulation (Tyler et al., 2015), acting like optic driving or unconscious steady state potentials. However, although sparse, some accounts with intracranial recordings ensure that tACS indeed reaches the parenchyma (Opitz et al., 2016; Ruhnau et al., 2017). Even a sub-threshold dose of tACS can in fact still be highly effective in a hormesis perspective (Giordano et al., 2017).

As for the way tACS produces its effects, it is under debate whether entrainment is the mechanism of action behind transcranial simulation. Rather than a forced alignment of endogenous oscillators, as we mentioned, tACS has been demonstrated to affect mechanisms of basic plasticity, like synaptic strength (Vossen et al., 2014; Podda et al., 2016) or perhaps even changes in astrocytes (only shown for direct current stimulation; Monai et

al., 2016). Phase synchronization is missing in non-invasive (Ruhnau et al., 2016a) and invasive in vivo recordings (Opitz et al., 2016; Lafon et al., 2017). However, many studies report ongoing performance or signal entrainment following tACS phase (e.g. Neuling et al., 2012a). Convincing evidence for tACS entrainment and its direct impact on behavior also comes from work on manipulation of individual oscillatory cycles (Cecere et al., 2015; Vosskuhl et al., 2015) and on network phase coupling (Polanía et al., 2012).

1.5 **Concurrent tACS & M-EEG**

Recently, some research groups have attempted to separate the massive artifact of current stimulation from real brain activity, as they are several orders of magnitude apart in terms of amplitude in encephalographic recordings. The tACS and EEG study with lucid dreaming facilitation mentioned above (Voss et al., 2014) tried to cancel stimulation noise with two simple procedures: first, by subtracting from EEG channels the signal of two reference electrodes adjusted for phase and amplitude; then, by using a band-stop filter at the stimulated frequencies to eliminate the residual artifact in the signal. This common practice naturally makes signal at the stimulated frequency band, which is likely a key element in the data, inaccessible.

Other groups came up with a solution to modulate a frequency of interest by stimulating another: the amplitude envelope (Hilbert transform) of a high frequency can follow the trend of a slower cycle and entrain that lower frequency. Therefore, a gamma stimulation that has its Hilbert envelope shaped like an alpha oscillation will induce effects in the alpha band, using another aspect of the cross-frequency mechanism explained above. In this way, the stimulated frequency can be notch filtered away, maintaining the relevant frequency and its modulation (Herring et al., 2017).

In another finding of behavioral entrainment cited above (Helfrich et al., 2014a), researchers were able to preserve the stimulation frequency by removing components, identified by independent and principle component analyses, expressing the remaining stimulation artifact after a sliding average subtraction window. They showed an online power increase at the stimulated alpha frequency, higher also than the offline power aftereffect.

Using EEG to record during tACS has however notable drawbacks. Both stimulation and EEG electrodes are attached to the scalp, which is very conductive and can limit the tACS intensity that can be used. Clipping of the signal, which precludes brain activity to be extracted, can originate if the recording channels saturate. This can be avoided with expert tweaking, like electrodes positioning, and techniques to increase scalp conductivity and reduce impedance (Helfrich et al., 2014b). Another problem is that some EEG electrodes cannot reach areas of the scalp covered by the tACS patches, which often are quite large, around 7 by 5 cm.

On the theoretic side, power increase at the stimulation frequency in the described studies (Voss et al., 2014; Helfrich et al., 2014b) could still be a residual artifact. None of these studies has demonstrated tACS modulatory effects of brain oscillations during stimulation, which could confirm a reliable separation of brain signals from the artifact. Even so, directly removing by filtering (Voss et al., 2014) or by subtracting (Helfrich et al., 2014b) a modeled version of the frequency artifact from the signal will also erode entrained brain oscillatory activity aligned to the tACS phase.

A different trend of approaches used to separate artifactual and physiological components is by renouncing on analyzing M-EEG channel data altogether and instead employ spatial filters to try to localize the sources of the signal, with artifacts confined outside the brain and proper cerebral activity inside.

Beamforming is a spatial filtering technique used in signal processing to gain spatial discrimination in any type of multi-sensor receiver array. In recent years, M-EEG studies have increasingly been employing beamforming techniques to localize neural activity in the brain (van Veen et al., 1997). Source reconstruction has the advantage of extracting artifactual elements of the signal in addition to projecting results into tridimensional space. Beamforming, in particular, is an adaptive procedure that selects the filters that best restrict covariant signal form surface recordings to one source, localizing and spatially separating activity in the brain. This means that beamformer filters silence sources of noise correlated across sensors and suppress external artifacts in every location (Brookes et al., 2008). This seems tailored to get rid of the tACS artifact, which is highly correlated noise. The first study to show the possibility of isolating cerebral activity in MEG during current stimulation used synthetic aperture magnetometry, a popular nonlinear beamforming method (Soekadar et al., 2013). Several other research groups resorted to beamforming to separate the stimulation artifact from brain activity (Strüber et al., 2014; Helfrich et al., 2014b; Witkowski et al., 2016; Herring et al., 2017).

It appears that power across every frequency band increases in reconstructed sources of activity during tACS, with peaks at the stimulated frequency and its harmonics. This could be inaccurate, and a consequence of a subset of MEG channels (the ones right above the tACS electrodes; Soekadar et al., 2013) having a stronger power change relative to the others and throwing off the result of the beamformer algorithm. Source-level signal beamformed with filters obtained separately from conditions with different tACS parameters will therefore show this variation in power. Regardless of this potential additional level of noise after reconstruction, source data can still be used for meaningful contrasts.

1.5.1 *Non-linear Artifacts and Noise*

A recent study (Noury et al., 2016) warns about possible non-linear interactions of heartbeat and respiratory signals with tACS. The artifact rejection approaches mentioned above are not able to remove completely these side artifactual components, and the sanitized data could still be contaminated with them. This is similar to the cross-frequency interactions mentioned above, but with uncontrolled and adverse effects. However, this perspective and the methods used in this study have been challenged (Neuling et al., 2016). If heartbeat and respiration, or any other nuisance variable that can influence the signal, vary constantly between conditions and participants in the study, only then this can be a problem. Even in that case, the effect will be weak, as the frequency of these physiological rhythms differs between individuals.

Source reconstructed signal shows unaffected inter-trial phase coherence at the simulation frequency (Ruhnau et al., 2016a), but a substantial increase in its harmonics. Again because of cardioballistic or respiratory movements and impedance changes (Noury et al., 2017) or just high variability in sensor signal (not only in power as noted by Soekadar et al., 2013, but also in phase consistency), the beamformer may not be able recovered the phase of the simulation frequency exactly in brain signal. This is especially a problem since entrainment is still on dispute as the mean tACS creates its effects, online or offline (as mentioned above).

Unfortunately, signal-to-noise ratio (SNR) in the MEG will indeed generally decrease while applying tACS. Electrical stimulation interacts not only with physiological signal, but also with movement, especially in the MEG, and movement interacts with biophysiological signal too. Unless the movement is consistent with the pacing of the experiment or of different conditions, it will not show up in the contrast, only decrease statistical power.

1.6 **Networks to be Aware.**

Neural correlates of consciousness (NCCs; Crick & Koch, 2003) are considered a recent undertaking of neuroscience, but they have been, in one way or another, the pinnacle of the scientific, psychological and philosophical enterprise since its beginning. Proper NCCs are defined as the elementary neural components that are minimally sufficient for a conscious experience (Crick & Koch, 2003). In neuroscientific research, NCCs are mostly treated as correlates of conscious access and reportability (Dehaene & Changeux, 2003), a more practical and operational conception (Franken & Slors, 2014). Disentangling processes that are mere consequences and not essential for the conscious event poses a serious difficulty (Palva et al., 2005; Aru et al., 2012). The prerequisites of conscious perception (NCC-pr; de Graaf et al., 2012; Aru et al., 2012) are distributed processes and network states that instead precede NCCs. They do not contain stimulus information, yet they are by definition necessary for an upcoming sensory stimulation to become reportable.

The strength of early responses does not predict conscious perception (Dehaene et al., 2006; but see Noy et al., 2015), so a robust sensory representation is not sufficient to *ignite* a parieto-frontal propagation, considered necessary for the information to become conscious (Dehaene & Changeux, 2003). The percept also needs to be connected first, but the way these connections are defined is not yet clear. The NCC-pr should then be the focus of research on consciousness, as they are the base on which this is determined. NCC-pr should be better outlined before confronting and interpreting other key contrasts (de Graaf et al., 2012; Ruhnau et al., 2014; Gallotto et al., 2017). Brain activity and network connections -qualifying a stimulus as perceived or not- also frame the broad cerebral system in which consciousness is shaped.

In most research, the ongoing brain fluctuations before the onset of the stimulus have been conventionally treated as noise, with researchers considering prestimulus activity irrelevant and averaging it away. However, the ongoing endogenous activity in the alert and conscious brain, an active and adaptive system continuously self-generating variability and predictions, will likely carry information. Studies found that conscious access inversely correlated with ongoing alpha power and phase in posterior sensory regions (for reviews, see: Ruhnau et al., 2014 and Gallotto et al., 2017). Prestimulus local alpha modulation is in line with the *gating-by-inhibition* hypothesis (Jensen & Mazaheri, 2010; VanRullen, 2016), stating that information flow is functionally controlled by posterior alpha oscillations, which alter cortical excitability in earlier regions that process unnecessary and unattended elements. Power and phase of the alpha rhythm are both controlled by prefrontal cortex with long-range top-down coupling (Bonnefond & Jensen, 2015). Alpha phase is also thought to provide a temporal code to organize sequential processing of sensory representations (at least in the visual stream; Jensen et al., 2014; Cerere et al., 2015; Michalareas et al., 2016; Coon et al., 2016).

1.6.1 *Coupling Theories*

In another hypothesis, information is likely to propagate in phases of coherent depolarization, when network nodes are coupled and neurons fire synchronously (*communication-through-coherence* hypothesis; Fries, 2005). Dynamical connections, continuously reshaping functional networks, are of great importance in determining the *readiness* or the *conscious* state of the cerebral system, or any brain state for that matter (Hutchison et al., 2013). Network connections in ongoing activity are thus also enabling sensory representations to propagate in interregional reentrant loops and to become an instance of consciousness.

Local activation patterns, like alpha oscillations in sensory regions, accompany particular functional connection changes. Although the alpha rhythm likely plays a role in network-driven excitability (following the theory of functional *gating-by-inhibition*; Jensen & Mazaheri, 2010), higher alpha power does not necessarily follow or precede increased interregional communication or enhanced connectivity. Slow and distributed cortical potentials around 4 Hz are also believed to be a frequency signature of subjective consciousness (He & Raichle, 2009; Li et al., 2014). Similarly, theta phase in the medial temporal lobe modulates neuronal firing in the gamma band correlated to conscious perception (Rey et al., 2014). Consciousness does not necessarily involve a specific frequency band, especially in a framework based on network dynamics and information transfer that does not make predictions on local activity. Oscillatory activity still likely carries and sustains conscious representations and their convergence (Siegel et al., 2012). A balance of neuronal rhythms in several frequency bands probably characterizes the NCC-pr (Gallotto et al., 2017).

Coupling in different brain regions, reflected in linear relationships and synchrony of their oscillatory phase, can be assessed with phase-locking and coherency measures. Additionally, neural codes and communication can be quantified using measures of information theory, which can describe and weight the amount of information and its transfer (Bastos & Schoffelen, 2015). Complex networks of information exchange can instead be studied with graph theory, which evaluates different aspects of the nodes of a network and their interconnections, like their clustering, efficiency or local and global organization (He & Evans, 2010). Network measures can characterize organizational dynamics and they can provide a better understanding of the intricate system that is the brain (Sporns, 2013). These analyses can be applied to single trials of encephalographic recordings, and are able to detect patterns of synchronized activity in the whole brain.

Network coupling between sensory and higher-level regions disposes the potential for propagation for the stimulus representation, or for only some of its aspects. For example, ongoing connectivity supported by beta oscillations in frontal and parietal areas is correlated to diverging conscious experiences of the same ambiguous upcoming stimulus (Hipp et al., 2011). Functional connections create optimal temporal windows in which

representations can become conscious, but the same content is suppressed if the stimulus falls outside. In this sense, brain network dynamics, establishing pathways along the nodes in sensory and fronto-parietal regions, can be said to form *windows to consciousness* (the expression used by Weisz et al., 2014, and similar to Rey et al., 2014).

Local excitability -reflected in low frequency power and phase- is essential to form a coherent representation of the stimulus in sensory areas, which is required -but not enough- for it to reach conscious awareness. Other predictors of conscious perception are found in networks, predisposed to integrate with higher-level regions the sensory information in the moment it arrives (Weisz et al., 2014; Ruhnau et al., 2014).

1.6.2 *Networks Effects of tACS.*

In order to force coupling in interregional network activity, one of the aforementioned studies on working memory (Polanía et al., 2012) stimulated two different regions independently with theta oscillations that were in phase or out of phase. This approach successfully illustrates the usage of network models to inform the most effective stimulation parameters. The authors recreated, with a 3-electrodes montage tACS, a fronto-parietal network synchronization in the theta band that was previously observed to improve memory performance. Gamma and beta interhemispheric coherence in visual cortex, along with motion perception, was also influenced by using two sets of electrodes and applying phasic and anti-phasic stimulation focally (Strüber et al., 2014; Helfrich et al., 2014b).

A couple of studies based on inter-individual frequency differences are also based on the theory of slow frequencies mediating the transmission of cortically-encoded representations multiplexed in the gamma activity. This information is contained in neuronal firing reflected on the envelope of gamma oscillations, which, depending on the process involved, are nested on some fundamental slow frequencies (Thut et al., 2012; Coon et al., 2016; Michalareas et al., 2016). As mentioned before, crossmodal interaction in visual-auditory illusory perception can be shifted in time by shortening or stretching the individual alpha cycle with tACS (Cecere et al., 2015). Similarly, the individual theta rhythm and working memory performance can be modulated with tACS, following the same rationale (Vosskuhl et al., 2015).

However, in all these cases except Polanía et al. (2012), the tACS could still be acting only on local ongoing activity, which has been shown to influence subsequent perception (Busch et al., 2009).

1.7 **Prying the Windows to Consciousness**

What does tACS do in the brain and how does it affect conscious perception? Alternate-current stimulation increases oscillatory activity involved in network dynamics and alters cognitive processing, but the direction of these effects is not always predictable (Veniero et al., 2017). This could be due to the nonlinearity of tACS interactions (Roberts & Robinson, 2012) and their state-dependent nature (Neuling et al., 2013), but also network interactions (Alagapan et al., 2016).

For example, since an increase in alpha or theta, frontally or locally driven, inhibits activity in sensory regions (Jensen & Mazaheri, 2010), alpha or theta stimulation over visual cortex reduces performance in visual perceptual tasks (Brignani et al., 2013). However, in the study of Helfrich and colleagues (2014b), accuracy fluctuated following occipital alpha tACS phase, but it increased overall relative to sham. As an explanation, the authors propose that alpha stimulation suppresses irrelevant information in visual processing (Bonnefond & Jensen, 2015). Because of state-dependence, we expect that tACS could be either facilitatory or inhibitory depending on the task. Distinctive neural populations overlap and they are sensible in different ways to stimulation depending on their current state (Silvanto et al., 2008) and on their arrangement in the cortical tissue (Miranda et al., 2006; 2013).

To cause this improvement in visual performance, tACS could also be affecting interregional communication by sustaining local coherence in sensory or in parieto-occipital areas. The increase in network coupling depends on coherent connections, which are formed by reverberatory synchronous activity (Fries, 2005). This endogenous pre-Hebbian mechanism is similar to the one that creates resonance and entrainment in tACS. Thus, it is possible that, as ongoing network dynamics influence the impact of neurostimulation (state-dependent effects), tACS can prompt connectivity changes (Ali et al., 2013). The local increase in coherence - localized in the middle of the two stimulation electrodes- could influence network synchrony, but not necessarily cause it. Similarly, behavior entrainment does not confirm a network interpretation of tACS effects, as it could act only through local excitability. These instantaneous phase-driven behavioral modulations make the whole network picture a little more complex if we take into account this temporal variability of information cascade (Jensen et al., 2014).

Given the wide range of applications tACS has, these are questions of outmost importance. We blindly stimulate ongoing brain oscillatory activity, an important determinant of cognitive processes, unaware of the mechanisms underlying the physiological and behavioral effects of tACS. It is likely that cortical network interactions are more complex than simple local and interregional synchronization, yet a basic investigation should still be possible with neurostimulation.

If we investigate the neural signal during tACS, we will be able to see how brain states and tACS interact and observe the physiological response in different conditions. This is a general question that needs to be addressed, which we can now do with concurrent tACS and M-EEG. More specifically, we can test whether there is an increase in local and interregional coherence during perceptual tasks and stimulation, even if tACS is localized in sensory areas (assuming that effects are focal; Kanai et al., 2010, 2013). By comparing measures of systemic synchrony and interareal coupling for different trials in which a stimulus falls in different stimulatory phases and it is processed differently, we could also see the efficacy of slow cortical rhythms in restraining propagation of information in the cortical network.

There is no need to know exactly what happens in the split-second of TMS stimulation to define causal links between brain states, cortical regions, processing time, cognition and behavior (Silvanto et al., 2008) and with TMS researchers can still assess causality, but for now this is a blind inference with no transparency: what actually happens to neuronal populations hit by the electromagnetic pulse is currently only speculative.

Conversely, with new methods combining MEG and tACS, a mechanistic account of brain stimulation appears more accessible. Even a less incomplete description of the mechanisms would trigger predictive knowledge potentially capable of generating new applications and treatments. Additionally, recording ongoing activity while probing it with artificial signal can contribute to the understanding of natural endogenous brain signal and its physiological basis. The ability to perturb a system is an additional tool that can be used to explore its mechanisms, and, in the case of tACS, we can investigate spontaneous cerebral oscillations.

1.7.1 *Course of the Project*

The technique from our group that opened up this line of research is described in the next chapter. It is a feasibility study demonstrating the recovery of endogenous and stimulus-driven brain modulations from MEG recordings during concurrent tACS (Neuling et al., 2015).

This data sparked another two investigations regarding the nature and the state-dependency of the influence of tACS. One of these studies, described in *Chapter 3*, examined the relationship between the tACS signal and the source data. It evaluated whether tACS entraining effects were as instantaneous as theorized, or rather reflected conduction delays, speculatively due to tissue connectivity. The association to different brain states of these effects was also observed (Runhau et al., 2016). This prompted another exploration of tACS state-dependency, whether it was observable in online data and which characteristics of ongoing activity could explain it. This study, reported in *Chapter 4*, further explores the nature of entrainment, but, most importantly, it connects tACS-related power changes to the state of brain functional networks.

Alternate current stimulation was then used in the context of near-threshold conscious perception. As network dynamics frame the propensity of sensory neural ensembles to affect downstream regions upon stimulus arrival (Ruhnau et al., 2014), we wanted to see how tACS affected these networks and the prestimulus predispositions to perception that depend upon them. In the framework in which we operate, graph theoretical metrics operationalize the moment-to-moment network-level reorganization, which factors in the awareness of an upcoming near-threshold stimulus (Weisz et al., 2014; Leske et al., 2015; Frey et al., 2016). This research lacked a causal interpretation of these oscillatory network dynamics toward conscious perception, which neurostimulation could provide. *Chapter 5* presents preliminary results of an ongoing near-threshold MEG study with concurrent prestimulus tACS.

We finally discuss the main findings, their contributions, implications and limitations, trying to reconcile our approaches with ongoing research on neural oscillations and brain stimulation. This initial effort in combining these different frameworks will be of great support in modeling and therefore predicting and controlling neurostimulatory effects, as well as unraveling the outreaching potential of this technique.

Chapter 2. **Feasibility of Concurrent MEG and tACS**

The work reported here was published as: Neuling, T., Ruhnau, P., Fuscà, M., Demarchi, G., Herrmann, C. S., & Weisz, N. (2015). Friends, not foes: magnetoencephalography as a tool to uncover brain dynamics during transcranial alternating current stimulation. *NeuroImage, 118*, 406-413.

2.1 **Abstract**

Brain oscillations are supposedly crucial for normal cognitive functioning and alterations are associated with cognitive dysfunctions. To demonstrate their causal role on behavior, entrainment approaches in particular aim at driving endogenous oscillations via rhythmic stimulation. Within this context, transcranial electrical stimulation, especially transcranial alternating current stimulation (tACS), has received renewed attention. This is likely due to the possibility of defining oscillatory stimulation properties precisely. Also, measurements comparing pre- with post-tACS electroencephalography (EEG) have shown impressive modulations. However, the period during tACS has remained a black box until now, due to the enormous stimulation artifact. By means of application of beamforming to magnetoencephalography (MEG) data, we successfully recovered modulations of the amplitude of brain oscillations during weak and strong tACS. Additionally, we demonstrate that also evoked responses to visual and auditory stimuli can be recovered during tACS. The main contribution of the present study is to provide critical evidence that during ongoing tACS, subtle modulations of oscillatory brain activity can be reconstructed even at the stimulation frequency. Future tACS experiments will be able to deliver direct physiological insights in order to further the understanding of the contribution of brain oscillations to cognition and behavior.

2.2 **Introduction**

Normal cognitive functioning requires a temporally precise coordination of neuronal ensembles at relatively local scales as well as over long distances. It has been proposed that brain oscillations play an essential role in these synchronization processes, and failures of the mechanisms enabling precise synchronization have been implicated in disordered cognition and psychiatric disorders (Herrmann & Demiralp, 2005; Schnitzler & Gross, 2005; Uhlhaas et al., 2008). However, the majority of literature relating oscillatory brain processes and behavior, in humans in particular, has been correlative. Recently, an increasing number of studies have employed brain stimulation techniques to *entrain* brain rhythms at natural frequencies to probe the effects on

behavior. Contrary to conventional neuroscientific experiments, the entrainment approach claims to use brain activity as the independent variable and behavior as the dependent variable allowing for more causal inferences. Most common techniques are transcranial magnetic stimulation (TMS; Thut & Miniussi, 2009) and transcranial alternating current stimulation (tACS; recently reviewed in Antal & Paulus, 2013; Herrmann et al., 2013; Marshall & Binder, 2013). Knowledge about the mechanism of action of tACS mainly stems from animal research: although the effect of tACS on single neurons is small, the rhythmic structure of tACS is able to modulate neuronal networks (Fröhlich & McCormick, 2010; Reato et al., 2010). Comparing pre- and post-tACS interventions, recent electroencephalography (EEG) studies have shown successful modulations of amplitude, phase, and coherence of oscillatory brain activity (e.g., Marshall et al., 2006; Neuling et al., 2012a, 2013; Polanía et al., 2012; Zaehle et al., 2010). These offline effects have clinical relevance, because of the opportunity to induce long-term changes of dysbalanced brain activity (Kuo et al., 2014). To extend this offline approach and to make a fully convincing case of the impact of tACS on brain activity, scientists need to be capable of uncovering the electrophysiological brain dynamics during stimulation (Herrmann et al., 2013). This would also allow disentangling online entrainment effects and aftereffects (Vossen et al., 2014). Measuring brain activity during tACS has, however, proven to be a challenge, due to the enormous artifact, several orders of magnitude higher in amplitude than the brain signal, introduced during electrical stimulation. In a worst-case scenario, this would cause a clipping of the signal, prohibiting separation of the artifact and the brain signal from the outset.

Regarding the latter issue of separating stimulation artifact from brain activity, recent EEG studies have made important advances. Helfrich et al. (2014b) utilized average subtraction and independent and principal component analysis (ICA/PCA) to remove the stimulation artifact and demonstrated online effects of tACS, among others the recovery of evoked potentials and an increase of spectral power at the stimulation frequency. Voss et al. (2014) subtracted the signal of a reference electrode and notch filtered the remaining signal centered on the stimulation frequency to show online effects. Critically, neither study demonstrated modulations of oscillatory brain activity during tACS (in particular at the actual stimulation frequency which a priori is a frequency of interest), only that oscillatory brain activity was different during tACS as compared to before or after stimulation. This distinction is crucial, because the former is a foundation for future studies utilizing tACS, especially for adaptive tACS protocols.

Although the first steps towards monitoring electrophysiological effects online during tACS have been taken, EEG poses important disadvantages: for example, the stimulation electrodes are attached to the highly conductive scalp, just like the recording electrodes that capture the artifact. The electrical coupling between

the electrodes severely limits the intensity at which tACS can be applied (Helfrich et al., 2014b; Voss et al., 2014), posing experimental design limitations and diminishing tACS efficacy (Fröhlich and McCormick, 2010). In our experience, various factors (e.g., distance of electrodes, skin conductivity, electrode impedance) can cause clipping to happen at intensities as low as .1 mA (even though under favorable conditions 1 mA can be reached; see Helfrich et al., 2014b). Another disadvantage is that the stimulation electrodes can cover a large area of the scalp (one electrode is usually 7 by 5 cm), which cannot be covered by EEG electrodes. This leads to reduced spatial sampling of the signal. Importantly, it has to be pointed out that while the current approaches (Helfrich et al., 2014b; Voss et al., 2014) based on EEG data of separating brain signals from artifact have shown a general increase of power at the stimulation frequency during tACS as compared to a pre-tACS period, it cannot be excluded with certainty whether these are still related to residual artifacts. The demonstration of an experimental modulation of power at the stimulation frequency during tACS is required to build a case that electrophysiological signals can be monitored during tACS. The evidence has not been provided so far.

Apart from *hardware* aspects, the reconstruction of oscillatory brain activity during tACS adds a further challenge: if the phases of the brain oscillations and the tACS signal align, previously used simple subtraction (e.g., Helfrich et al., 2014b; Voss et al., 2014) will cancel the artifact along with the to-be-analyzed brain activity. Here, we demonstrate that magnetoencephalography (MEG), combined with advanced source imaging approaches, is capable of overcoming the aforementioned limitations. Our work is based on a recent demonstration of combined transcranial direct current stimulation (tDCS) and MEG (Soekadar et al., 2013). The authors utilized spatial filtering (synthetic aperture magnetometry) to suppress artifactual activity outside the brain in order to reconstruct and localize oscillatory activity inside the brain. It is an open issue whether, due to the high synchrony of the brain signal and the stimulation artifact, it would be possible to separate these two and uncover subtle modulations of brain activity during tACS. Using interventions that lead to well-established and robust modulations of alpha power (eyes open vs. closed and stimulus induced alpha power decrease), we demonstrate for the first time that MEG in combination with a similar spatial filtering technique as used by Soekadar et al. (2013) can be utilized to disentangle oscillatory brain activity from the highly correlated tACS signal. The possibility of studying brain activity, even at the stimulation frequency during the ongoing tACS, opens up new avenues for understanding tACS related effects on brain functioning with far reaching consequences for cognitive and clinical neuroscience.

2.3 **Material and Methods**

2.3.1 *Subjects*

Seventeen healthy volunteers (9 males, 28 ± 4 years old; all right-handed) without psychiatric or neurological disorders took part in this study. The experimental protocol was approved by the ethics committee of the University of Trento, and all participants gave written informed consent before the beginning of the experiment.

2.3.2 *Stimuli and procedure*

After applying the MEG coils and tACS electrodes to the head, the stimulation intensity was determined (see next section). Participants were seated in an upright position in the MEG room. Before the first block, a three-minute resting condition with the eyes open was performed. The data from this measurement were used to estimate the the individual alpha frequency (IAF) of the subject (see below). Three blocks with identical basic setup followed during which either sham, weak, or strong tACS was applied (see next section): in the first part of a block, subjects were asked to keep their eyes open for 2 min until a tone and a visual instruction presented on a screen asked subjects to close their eyes for another 2 min. This resting-state measurement was followed by a second part consisting of passive viewing and listening. 100 auditory and 100 visual stimuli were presented in random order, divided into two sub-blocks separated by a self-paced break. The visual stimuli were moving Garbor patches, subtending 0.46° of visual angle, with 3 cycles (resulting in 6.5 cycles/°), with a Gaussian envelope of .07°, and oriented at -45%, moving upward at 4.43°/s with randomized initial phase. The Garbor patches were projected centrally on a screen (62 × 35 cm, 1920 × 1080 pixel resolution, 120 Hz refresh rate) at 50% contrast and the background was a mean-luminance uniform gray. The auditory stimuli were pure tones of 1 kHz, sinusoidal, sampled at 44.1 kHz, and presented binaurally via air-conducting tubes with ear inserts. The duration of all stimuli was 2 s and the inter-stimulus interval was set to 2.5 ± 0.5 s (uniformly distributed) which amounts to 16 min per block. Since the participants experienced the sensation of suprathreshold stimulation during the threshold assessment (see below), we could ask them after each block to indicate whether they perceived the stimulation. In the end, three minutes of resting state (eyes open) without any stimulation was recorded. After the experiment, subjects were asked to fill out a translated questionnaire that captures the possible adverse effect of transcranial electrical stimulation (Brunoni et al., 2011).

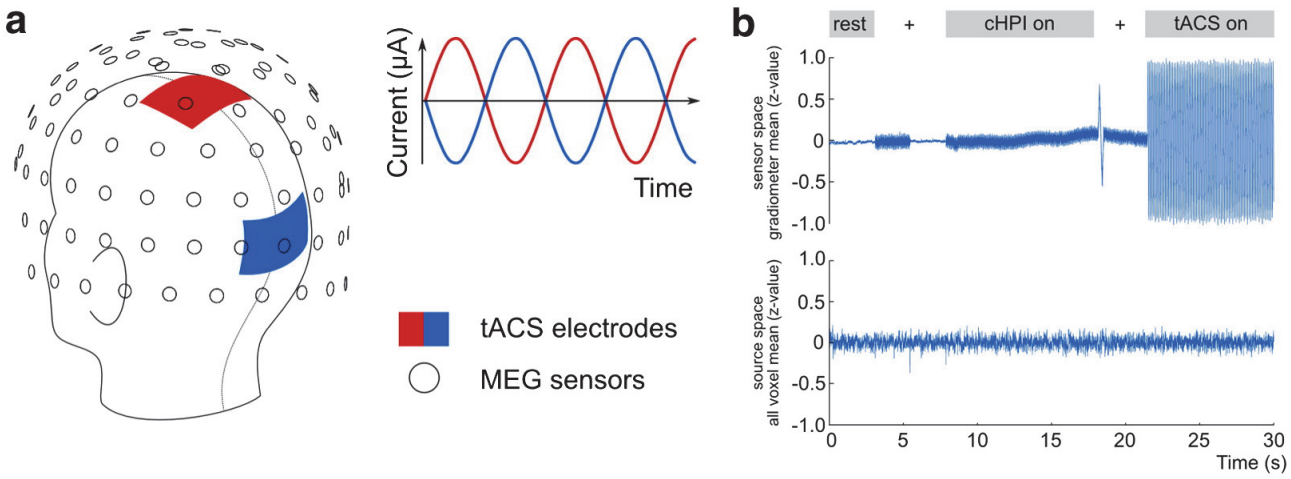


Figure 2.1 ***Experimental setup and stimulation artifact***
a: Schematic illustration of the electrode and MEG sensor positions: Stimulation electrodes were centered at Oz and Cz according to the international 10-20 system. *b:* Time series depicting the tACS artifact in sensor space (*upper*) and after transformation into source space (*lower*), both z-normalized. *b, upper:* During tACS (tACS on), the artifact is several magnitudes higher than the resting state brain activity (rest) and the signal emitted by the head positioning coils (cHPI on). Note the initial head position estimation signal (around 3-6 s), and the sharp transition at around 18 s corresponding to the impedance check of the tACS device. *b, lower:* The same time series in source space. Note the suppressed tACS artifact. For illustrative purposes, the time series in source space was band-pass filtered between 1 and 100 Hz.

2.3.3 *tACS parameters*

A battery-operated stimulator system (DC-Stimulator Plus, NeuroConn GmbH, Ilmenau, Germany) was placed outside the magnetically shielded room. It was connected to the stimulation electrodes via the MRI module (NeuroConn GmbH, Ilmenau, Germany). The stimulator delivered an alternating, sinusoidal current at the IAF via two conductive rubber electrodes (NeuroConn GmbH) centered at electrode positions Cz and Oz of the international 10-20 system (*Fig. 2.1a*). These positions were chosen for maximal stimulation intensity in the parieto-occipital cortex (Neuling et al., 2012b). The electrodes had a size of 7 by 5 cm and were applied with a conductive paste (Ten20, D.O. Weaver, Aurora, CO, USA) resulting in impedance values of 6.13 ± 0.8 kΩ (mean ± SE). The electrode cables were located on the right side of the participant's head. The stimulation intensity was kept below each subject's sensation and phosphene threshold in order to keep them naive regarding the stimulation condition (for individual parameters, cf. supplementary *Table S2.1*). To obtain the threshold, the subject was first familiarized with the skin sensation. The subject was then stimulated with an intensity of 400 μA (peak-to-peak) at 10 Hz for 5 s. The intensity was increased by steps of 100 μA until the subject indicated skin sensation or phosphene perception or an intensity of 1500 μA was reached. In the two cases in which the subject already reported an adverse effect at 400 μA, the intensity was reduced to a start level of 100 μA and increased by steps of 100 μA in line with the start level of 400 μA. The staircase procedure resulted in average stimulation intensities of 653 ± 447 μA (mean ± SD). As mentioned above, the experiment comprised three different stimulation blocks (sham, weak, and strong tACS). While the order of the first two

blocks (sham and weak tACS) was pseudorandomized, the strong tACS block was always the last block in order to avoid after-effects during the sham and weak stimulation blocks. During the sham block, the experimental setup was the same as in the other blocks, but no electrical stimulation was applied. A stimulation intensity of 50 μA was delivered at IAF during the weak stimulation block to induce an artifact, but likely without an effect on brain activity (Reato et al., 2013). The individual estimated threshold level minus 100 μA was used as stimulation intensity in the strong tACS block.

2.3.4 *MEG data recording*

Magnetic brain activity was recorded at 1000 Hz (hardware filters: 0.1-330 Hz) using whole head MEG (Elekta Neuromag Vectorview, Elekta Oy, Finland), spatially sampling the signals at 102 positions. Each position consists of a channel triplet of one magnetometer and two orthogonal planar gradiometers yielding 306 sensors overall. The MEG system is housed in a magnetically shielded room (AK3b, Vacuumschmelze, Germany). Fiducials (nasion and left and right pre-auricular points), the location of five head position indicator (HPI) coils and N=200 headshape samples were digitized prior to the experiment. These points served for later head modeling as well as for determining the head position within the helmet prior to each run. The latter controls for large head movements over the course of the experiment.

2.3.4.1 IAF determination

For each subject we acquired 3 min of resting state activity with the eyes open as first measurement. In order to determine each subject's IAF, these data were analyzed offline immediately after the measurement. First, continuous data were cut into segments of 2 s each, and frequency analysis (1 to 25 Hz, .25 Hz resolution, Hanning window, 4 s padding) was subsequently performed on each trial to estimate the power spectrum. Then we manually chose a few gradiometers showing a prominent alpha peak on the averaged trials, and assessed the frequency of the peak. Two subjects did not exhibit a clear alpha peak in the resting state data with the eyes open, and therefore we had to determine their alpha frequency on a subsequent resting state block with the eyes open and eyes closed conditions.

2.3.5 *Offline MEG data analysis*

2.3.5.1 Preprocessing

Continuous data were offline band-pass filtered between 1 and 200 Hz and downsampled to 512 Hz. Then the data were segmented into non-overlapping epochs of 2 s for the eyes open vs. eyes closed data or 2 to 3 s relative to stimulus onset for the auditory/visual stimulation condition, respectively. Noisy and dead sensors were identified in the sham block and excluded for the other two blocks (weak and strong tACS). Epochs containing artifacts (caused by, e.g., blinks/muscle activity) were removed from the stimulation free block. Since the

electrical stimulation leads to signals of several orders of magnitude higher than actual physiological data (see *Fig. 2.1b*), we refrained from removing further artifacts from the tACS blocks.

2.3.5.2 Source projection of raw data

Sensor level data were projected into source space using linearly constrained minimum variance (LCMV) beamformer filters (van Veen et al., 1997), which is a standard procedure in source analysis of electrophysiological data. We followed a procedure described here for single virtual sensors (http://www.fieldtriptoolbox.org/tutorial/shared/virtual_sensors) and extended it to 889 points covering the whole brain (see below). For this, epoched raw data were filtered from 1 to 40 Hz and the covariance matrix of each single trial was calculated and averaged across trials. Together with single-shell head models (Nolte, 2003) derived from the individual head shape and the lead field matrix, the covariance matrix was used to obtain the beamformer filters. These were subsequently multiplied with the sensor level time series to obtain time series for each source location. Using this process, we were able to perform identical analyses on the sensor as well as on the source level. A favorable feature, for our purposes, of beamformers is that they are geared to optimally estimate activity at a source point while suppressing activity originating from elsewhere. Importantly, beamformers effectively suppress noise sources that are correlated over sensors, which is evident in the case of tACS. We used a grid with a size of 889 points equally spaced by a 1.5 cm distance in Montreal Neurological Institute (MNI) space and warped these positions into individual head space. An MNI template brain was used for subsequent visualization purposes.

2.3.5.3 Resting-state spectral power

For the eyes open/eyes closed resting-state data, spectral power estimations (for both sensor and source space) were performed after Hanning-tapering the epochs (see *2.3.5.1 Preprocessing*), for frequencies ranging from 2 to 30 Hz in 1 Hz steps. Subsequently, averages of the power spectra were calculated for all stimulation conditions (sham, weak tACS, and strong tACS), and eyes open/eyes closed data per subject. For visualization purposes, the source space spectral power results for the eyes open vs. eyes closed conditions were then statistically compared in the alpha range (8-12 Hz) as described below (see *2.3.5.6 Statistical analysis*).

2.3.5.4 Stimulus-evoked responses

In sensor and source space (see *2.3.5.1 Preprocessing* above), epochs were low-pass filtered at 25 Hz and reduced to -0.2 to 1 s relative to stimulus onset. Then, all remaining epochs per stimulus (visual/auditory) and condition (sham, weak tACS, strong tACS) were averaged for each individual subject yielding evoked responses (ER) in sensor and source space. For sensor space data all epochs were baseline normalized by subtracting the average baseline (-0.2 to 0 s) amplitude from the whole epoch. In source space, baseline normalization was

accomplished by computing the absolute value of the signal (thus discarding potential polarity differences of a source across participants) and computing a relative change, i.e., by subtracting the average baseline amplitude from the epoch and dividing this difference by the average baseline amplitude. Using relative change as baseline normalization effectively abolishes the well-known depth bias of the beamformer (van Veen et al., 1997). For visualization purposes, the source space results for the auditory M90 (peak at 90 ms) and the visual M150 (peak at 150 ms) were then statistically compared as described below (see *2.3.5.6 Statistical analysis*).

2.3.5.5 Stimulus-induced power modulations

Spectral power estimation in sensor and source space in single epochs was performed on Hanning-tapered time windows from -0.5 to 2.5 s (in steps of 0.05 s) relative to the stimulus (visual/auditory) onset. The sliding window had a fixed length of 0.4 s. Frequencies of interest ranged from 2 to 30 Hz in steps of 1 Hz. Then, power estimates of trials belonging to the same stimulus and condition were averaged for each individual subject. In source space, stimulus related alpha band (8-12 Hz) suppressions in the time window of 0.3-1.5 s post-stimulus were compared against baseline. To do that we computed the normalized difference as described in the following paragraph.

2.3.5.6 Statistical analysis

Source space statistical results were accomplished in a similar manner for the resting state and the stimulus evoked/induced activity. We calculated the normalized difference (cf. Spaak et al., 2014) for each time/voxel point for the ER and each time/frequency/voxel point for the spectral estimates. The normalized difference is computed as (A - B) / (A + B). A and B corresponded to the eyes open and eyes closed, visual M150 and auditory M90, and post-stimulus alpha and baseline, respectively. Following a permutation test approach the normalized difference was computed both for the observed data and for all possible (131072) permutations of the above described conditions. Based on the per-voxel permutation distribution, we obtained individual probabilities for the observed data. All p-values were corrected for multiple comparisons across voxels using the false discovery rate procedure (FDR, Benjamini & Hochberg, 1995; Genovese et al., 2002).

Signal processing and statistical analysis were performed using the Fieldtrip toolbox (Oostenveld et al., 2011).

2.4 **Results**

To demonstrate that our approach is capable of uncovering subtle modulations of oscillatory brain activity during tACS, we combined non-invasive brain stimulation with concurrent MEG. We faced a challenge to disentangle oscillatory brain activity from the artifact caused by tACS, which is several magnitudes higher (*Fig. 2.1b*) and in the same frequency range (8-12 Hz) as the frequencies we aimed to analyze. One promising

technique to overcome the challenge is spatial filtering by means of LCMV beamforming (van Veen et al., 1997). Beamformers are especially suited to remove the tACS artifact because they suppress noise sources that are correlated over sensors. We used well-established interventions to modulate alpha power without tACS and during tACS, which was adjusted to the IAF and sensation threshold. We subsequently analyzed alpha modulations and compared the results in sensor space and source space.

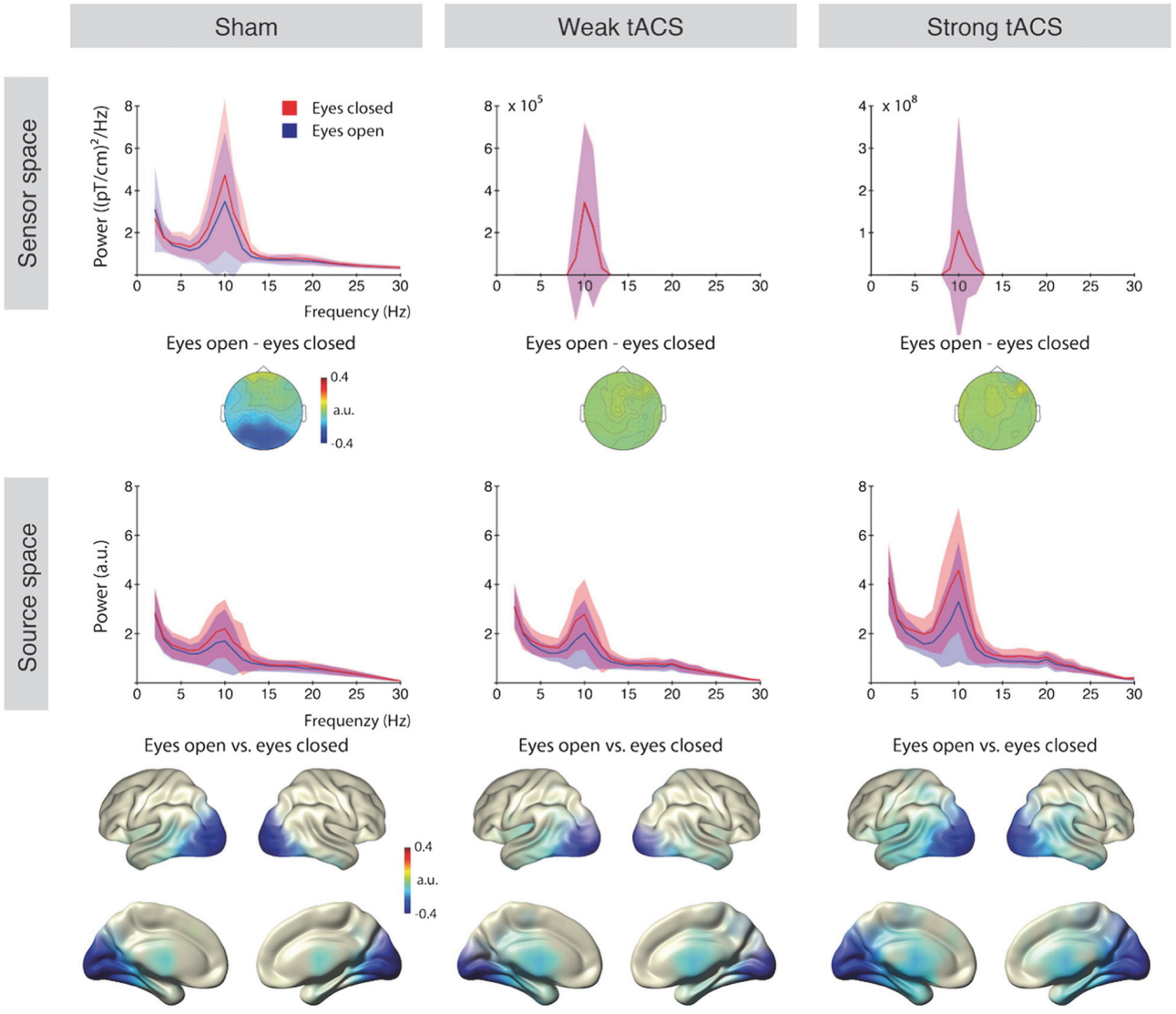


Figure 2.2 ***Resting state alpha modulations***
Top: Sensor level average spectra across all gradiometers (combined) in the three stimulation conditions. The typical increase of alpha band power when the eyes are closed is only observable without stimulation. Topographies show the difference of alpha power (8-12Hz) in the eyes open minus the eyes closed condition. Only without stimulation (sham), a parieto-occipital decrease is observable; under weak- and strong tACS the topographies reflect the stimulation artifact. Note the different scales in the spectra depending on the stimulation. *Bottom*: Source space spectra across all cortical sources. The typical increase of alpha band power when the eyes are closed is clearly observable in all stimulation conditions. The statistical maps show a contrast (normalized difference) of the eyes open vs. eyes closed condition; FDR corrected significant effects were observed in all stimulation conditions. For illustrative purposes the maps are thresholded at $p < 0.001$. Shaded areas represent the standard deviation. *a.u.* - arbitrary unit.

2.4.1 *Eyes open/closed related alpha modulations*

Modulations of cortical alpha power caused by closing the eyes were investigated in sensor and source space. In the sham condition, clear occipital modulations were observed in the alpha range (8-12 Hz) in sensor space. During weak and strong tACS, the sensor space data were completely dominated by the stimulation artifact, thus rendering any endogenous alpha modulation invisible (*Fig. 2.2*, top middle and right). Note that the scales in the spectra change drastically from sham to weak and strong stimulation (*Fig. 2.2*, top). Thus on a sensor level, the stimulation artifact completely covers neurophysiological effects (see also *Fig. 2.1b*). In source space, however, both weak and strong tACS showed spectra with similar morphology as the sham condition (*Fig. 2.2*, bottom). Statistical contrast revealed similar sources mainly localized to visual cortices along the calcarine fissure ($p_{FDR} < .05$). Interestingly, but not within the scope of this proof of principle manuscript, strong tACS seems to affect alpha power in both conditions, eyes open and eyes closed. This could be an indicator of entrainment on the neuronal level; however, we did not further investigate the effect at this point. For a demonstration that possible effects are not residual artifacts, see the supplementary *Fig. S2.1* for a comparison of two subjects that received weak and strong tACS intensities.

2.4.2 *Visual and auditory evoked responses*

Although not the focus of our interest, evoked activity time-locked to visual (Gabor gratings) and auditory stimuli (sine tones) was investigated in sensor and source space. In the sham condition (*Fig. 2.3*, left),clear auditory and visual ER could be observed on sensor and source levels. Statistical contrasts of the visual M150 (peak at around 150 ms) and the auditory M90 (peak at 90 ms) demonstrated that the M150 was mainly generated in primary and secondary visual areas in the occipital cortex ($p_{FDR} < .05$), and the M90 was generated by sources in the superior temporal gyrus (including primary auditory cortex) and additional sources in the frontal cortex ($p_{FDR} < .05$). During weak and strong tACS, the sensor space data were completely dominated by the stimulus artifact without any clear ER being discernible (*Fig. 2.3*, top middle and right). Analogous to *Fig. 2.2*, please notice in *Fig. 2.3* (top panel) the drastic change in scales: even following averaging of ~100 trials, the ER in the strong tACS condition is still a factor ~250 times larger than the *real* ER. In dramatic contrast to the sensor level, source space analysis of weak and strong tACS conditions revealed clear modality specific ERs with strikingly similar time courses and spatial distributions as the sham condition (*Fig. 2.3*, bottom).

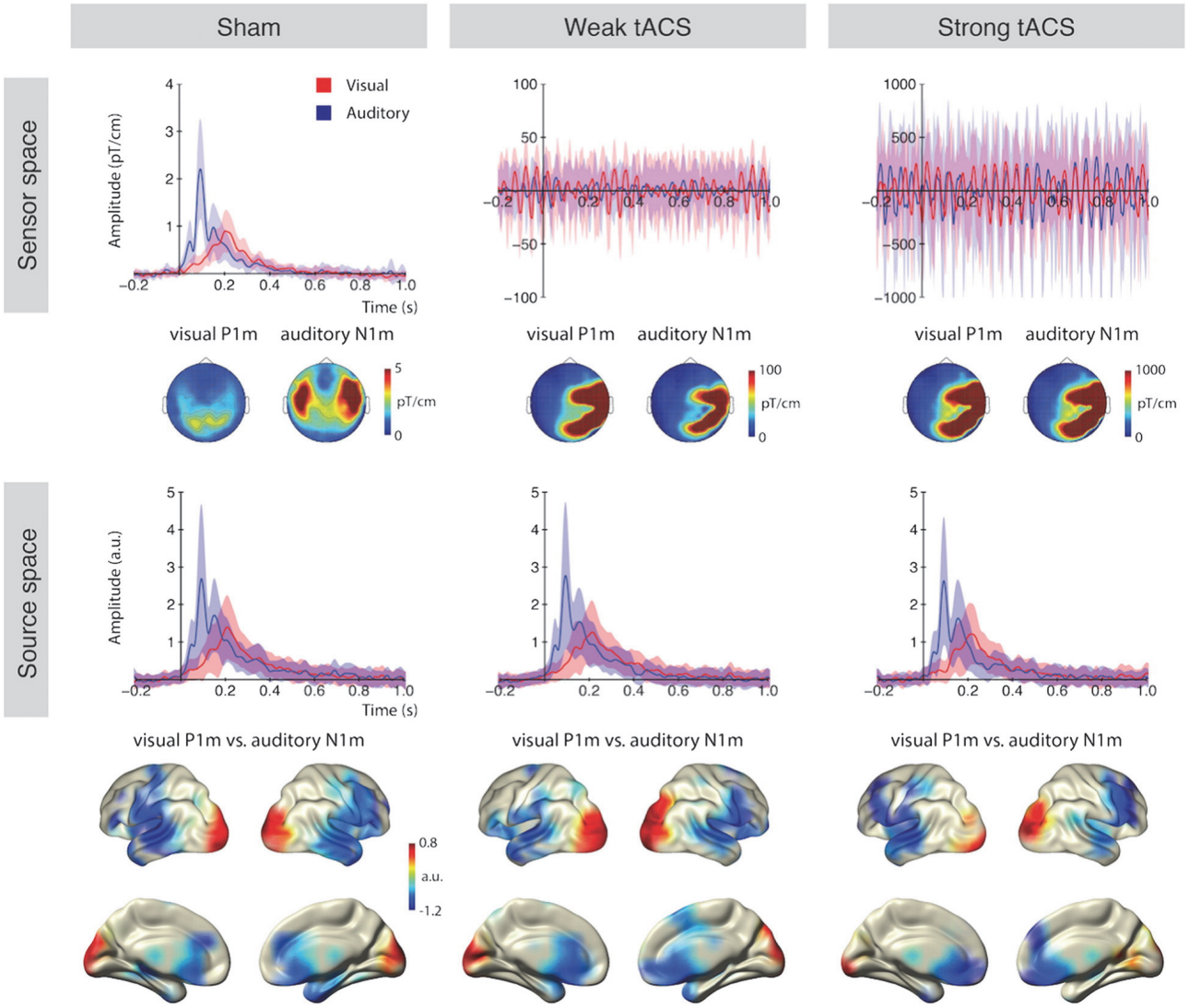


Figure 2.3 ***Event-related fields to visual and auditory stimuli***
Top: Sensor level average across all gradiometers (combined, which is the squared root-mean across gradiometer pairs) in the three stimulation conditions. Topographies show the visual M150 (30ms window around the peak at 150ms) and the auditory M90 (30ms window around the peak at 90ms). Modality specific ERs and topographies are observable only without stimulation (sham); under weak and strong tACS stimulation, the topographies reflect the stimulation artifact, especially on the right side where the electrode cables were located. Note the different scales in the three conditions. *Bottom*: Source space averages across all cortical sources. Visual and auditory ERs are clearly observable in all stimulation conditions. The statistical maps show a contrast (normalized difference) of the visual M150 (red colors) vs. the auditory M90 (blue colors); FDR corrected significant effects were observed for both modalities. Auditory source activity was stronger than visual source activity, thus, for illustrative purposes the auditory activity was thresholded at $p < 0.001$ and visual activity was thresholded at $p < 0.05$. Shaded areas represent the standard deviation. a.u. - arbitrary unit.

2.3.3 *Stimulus-induced alpha decreases*

Next, we investigated alpha power decreases induced by visual stimulation. In sensor space, the alpha decrease after stimulus onset was observable only in the sham condition (*Fig. 2.4*, top left). As expected, the alpha suppression, as evaluated with statistical contrasts of post-stimulus alpha power (8-12 Hz) vs. baseline, was

dominant in bilateral visual cortical regions ($p_{FDR} < .05$, *Fig. 2.4*, bottom left). With stimulation, the tACS artifact dominated the time-frequency representations making it impossible to observe any modulations in the alpha range. Even worse, due to the tACS artifact being constant over the course of the trial, the act of baseline normalization effectively removes any activity in the alpha range (*Fig. 2.4*, top middle and right). Time-frequency analysis on our source space data, on the other hand, yielded clear alpha decreases.

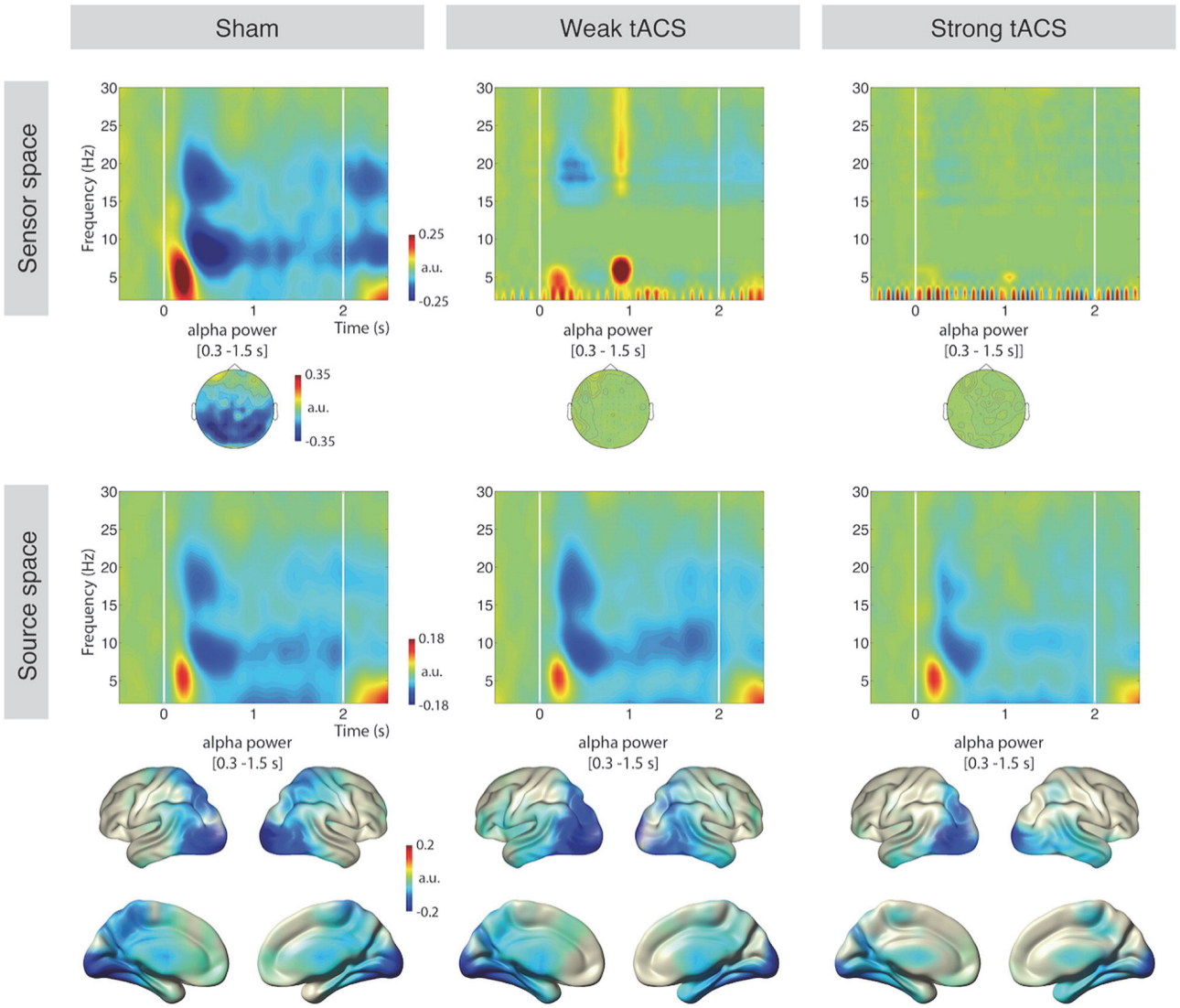


Figure 2.4 ***Alpha decrease induced by visual stimuli***
Top: Sensor level average across all gradiometers (combined) in the three stimulation conditions. Topographies show alpha band (8-12 Hz) activity during stimulus presentation (0.3-1.5 s). Parieto-occipital alpha desynchronization is observable only in the sham condition. The time-frequency representations are baseline corrected (relative change). Note, the strong signal at around 1s in the middle is a broadband artifact present only in one subject. *Bottom*: Source space averages across all cortical sources. Stimulus induced alpha decrease (normalized difference of post-stimulus alpha vs. baseline) is observable in all stimulation conditions in parieto-occipital areas. The statistical maps are thresholded at $p < .001$ for descriptive purposes, FDR corrected significant effects were observed in all conditions. *a.u.* - arbitrary unit.

Similar to the sham condition, statistical contrasts of post-stimulus alpha power (8-12 Hz) vs. baseline in the tACS conditions yielded dominant generators in posterior regions ($p_{FDR} < .05$, *Fig. 2.4*, bottom middle and right). On a descriptive level, the alpha suppression in source space appears less pronounced for the strong tACS condition compared to the two others, which could be an actual effect of the neurostimulation leading to increased inhibitory states (Klimesch et al., 2007). As addressing this issue was not the goal of the study, we did not follow-up this descriptive pattern.

2.5 **Discussion**

In this study, we demonstrated, for the first time, the feasibility of using MEG to uncover brain dynamics during tACS. It was not our intention to reveal actual immediate effects of tACS on oscillatory brain activity, but provide a critical proof of concept for future studies. The essential component of our approach is the successful removal of the stimulation artifact to uncover subtle modulations of oscillatory brain activity during oscillatory brain stimulation in the same frequency range. In order to provide a validation of our approach, we resorted to well-established paradigms known to elicit very robust alpha modulations. As expected, in the sham condition robust ERs and alpha modulations (both for eyes open vs. eyes closed and stimulus-induced power decrease) were obtained on the sensor as well as at source level. However, already at weak stimulation, sensor level data were utterly unusable, with the artifact dominating all results. Importantly for the resting state data, even the statistical contrast between eyes open and eyes closed did not remove the *common* influence of the artifact, yielding no meaningful difference between the conditions during real tACS. Also, since tACS was constant throughout one block, baseline normalization in the time-frequency analysis effectively removed any traces of alpha modulations following auditory and visual stimulation. In contrast to the sensor level, source level analysis provided strikingly similar patterns to those obtained for the sham condition. Not only did we obtain the well-established ERs, we also showed the known alpha modulation patterns, i.e., power increases during eyes closed and power reductions during sensory stimulation. Importantly, the results presented in *Figs. 2.2-2.4* show contrasts. That means that even if there is contamination by the tACS it should be similarly affecting both conditions (be it eyes open vs. closed or sensory stimulation). Thus, the crucial comparison is not the curves but the statistical parametric source maps below the curves. Overall, our study goes significantly beyond a previous EEG approach, which for alpha tACS showed alpha increases only relative to a condition without stimulation (Helfrich et al., 2014b; see Voss et al., 2014, for a study in the gamma frequency range).

An additional advantage of our beamforming approach is that it can be applied objectively, whereas the approach introduced by Helfrich et al. (2014b) relies on subjectively removing ICA components for each subject. As suggested by Schmidt et al. (2014), it is of utmost importance for future studies to measure brain

activity and brain modulations during tACS stimulation. This is particularly important for the stimulation frequency itself, which is at the center of interest in most tACS studies. Showing that alpha modulations can be faithfully obtained while undergoing alpha tACS (at intensities common in the literature) is the most important contribution of this study.

The critical step in our analysis pipeline is the projection of the sensor data into source space using the LCMV beamformer, which allows for a separation of brain activity from the stimulation artifact even during high levels of tACS. This well established and frequently used source projection exploits a feature of beamformers, which suppress perfectly correlating data, since these are physiologically improbable (van Veen et al., 1997). As tACS introduces signals that perfectly correlate over basically all sensors, it is in principle well suited to remove the stimulation artifact. Our approach from a signal processing perspective is not innovative and very similar to the one reported by Soekadar et al. (2013), who showed it was feasible to remove artifacts introduced by tDCS. However, as long as sensors do not saturate, high-pass filtering may already be sufficient in the case of DC stimulation, whereas in the case of tACS filtering the sensor level data would lead to an unacceptable loss of information. While opening up new avenues to investigate experimental effects during neurostimulation, the potential feasibility of our approach may have been foreshadowed by other applications of beamformers in the case of time-varying artifacts. For example, Wong and Gordon (2009) demonstrated the successful use of beamformers for suppressing artifacts introduced by cochlear implants on EEG data. Furthermore, in a combined EEG/magnetic resonance imaging (MRI) measurement, beamformers were successfully used to cancel out the gradient and cardioballistic artifacts (Brookes et al., 2008). This shows that the presented beamforming approach is not limited to MEG and could also be realized with EEG, however, likely with greater problems (see *2.2 Introduction*) and less spatial accuracy.

Until now, human tACS studies mostly have relied on offline measurements of brain activity which required stimulation protocols of long duration that produce after-effects (Zaehle et al., 2010; Neuling et al., 2012a, 2013), or indirect measures that correlate with changes of brain activity were used, e.g., behavior or TMS triggered motor evoked potentials (Feurra et al., 2011; Neuling et al., 2012a; Polanía et al., 2012; Strüber et al., 2014). With our approach, it will be possible to demonstrate how tACS directly modulates oscillatory brain activity and subsequent behavior: future research will be capable of transporting cognitive neuroscientific experiments more straightforwardly in a combined MEG-neurostimulation setting. The same line of thought applies to the investigation of how entrainment may act on diverse disorders for which dysfunctional oscillations have been assumed to be critical, such as tinnitus (Weisz et al., 2005), schizophrenia (Uhlhaas & Singer, 2012), and Parkinson's disease (Hammond et al., 2007).

Finally, our approach allows the investigation of the impact of tACS directly on the human brain at an unprecedented level. This issue is currently poorly understood and relies on the extension of animal findings onto humans (Reato et al., 2013), which may be more or less valid. As an example, we will describe two unresolved and pressing problems with regard to tACS efficacy: dosage (i.e., duration and intensity) and interindividual differences (Krause & Cohen Kadosh, 2014; López-Alonso et al., 2014). Thus far, considerations to adjust the dosage of tACS have been rather elusive: stimulation was either applied with fixed parameters for all participants (e.g., Antal et al., 2008) to keep the dosage seemingly constant, or specific parameters were adjusted individually considering, for instance, endogenous frequency or sensation threshold in order to maximize tACS efficacy and enable sham control (Neuling et al., 2012a, 2013; Zaehle et al., 2010). There is only sparse evidence on how external stimulation parameters and internal parameters of the participant (e.g., brain state, individual oscillatory power and frequency) contribute to tACS efficacy (Fröhlich & McCormick, 2010; Neuling et al., 2013; Schmidt et al., 2014). In future studies, brain activity recordings during tACS, as presented here, will reveal the interplay of dosage, internal parameters, and efficacy. Furthermore, dosage control will have groundbreaking consequences for future therapeutic interventions that utilize tACS in order to enhance beneficial brain oscillations or suppress pathological brain activity (Fröhlich, 2014; Kuo et al., 2014), because it allows for patient-tailored stimulation protocols.

To conclude: until now, the progress of tACS in neuroscientific research has been hindered by the seemingly insurmountable challenge to analyze brain activity during stimulation, in particular at the stimulation frequency. In the present study, using well-established alpha effects, we have presented a proof of concept to overcome this limitation. This will allow unprecedented insights into understanding the online impact of tACS on brain function.

2.6 **Supplementary Material**

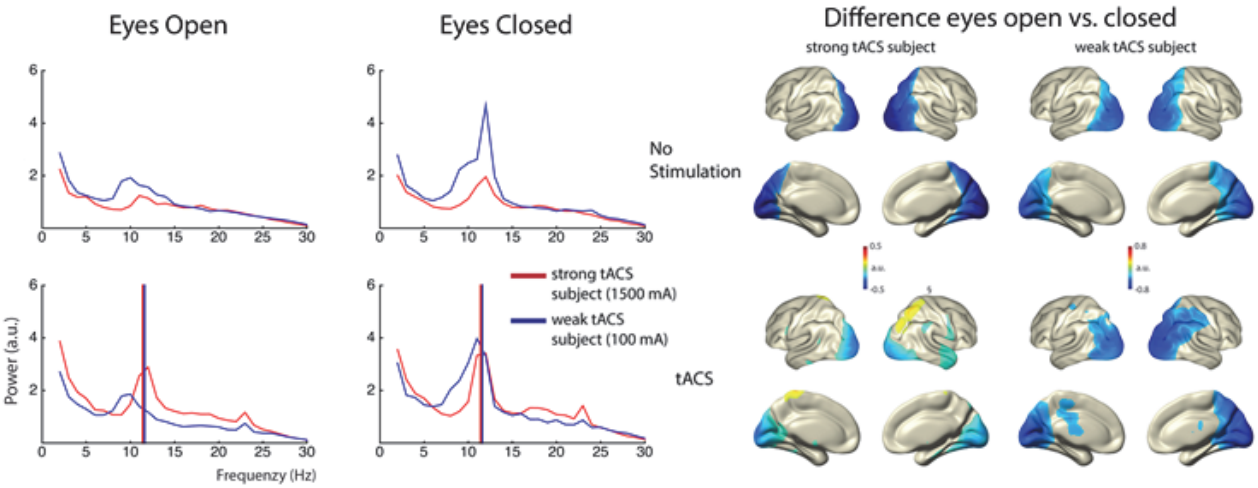


Figure S2.1 ***Artifact suppression in strong vs. weak stimulation***
Left: Power-spectra of two subjects that received low (100 µA) and high (1500 µA) tACS intensities. The vertical line depicts the stimulation frequency (11.5 Hz). *Right:* Normalized difference between the eyes open and the eyes closed condition in the respective subjects during tACS and sham stimulation. The top graphs and brain topographies depict contrasts in the no stimulation condition, the bottom ones in the tACS condition. *a.u.* - arbitrary unit.

Subject	IAF(Hz)	Int (µA)	Z (kΩ)
1	10	1000	4.3
2	9	600	2.8
3	10	400	4.3
4	10	500	9.0
5	10	900	14
6	10.5	600	9.8
7	10.5	500	2.4
8	10	400	4.0
9	10	1500	5.9
10	11	500	4.9
11	11.5	100	7.0
12	10	400	9.9
13	11.5	1500	6.7
14	10.5	200	4.2
15	10	300	3.9
16	10	300	6.1
17	10	1400	2.7

Table S2.1 ***Individual stimulation parameters***
IAF: individual alpha frequency, *Int:* stimulation intensity, *Z:* impedance.

Chapter 3. **Phase Coherence and its Dependencies during tACS**

The work reported here was published as: Ruhnau, P., Neuling, T., Fuscà, M., Herrmann, C. S., Demarchi, G., & Weisz, N. (2016). Eyes wide shut: transcranial alternating current stimulation drives alpha rhythm in a state dependent manner. *Scientific reports, 6*, 27138.

3.1 **Abstract**

Transcranial alternating current stimulation (tACS) is used to modulate brain oscillations to measure changes in cognitive function. It is only since recently that brain activity in human subjects during tACS can be investigated. The present study aims to investigate the phase relationship between the external tACS signal and concurrent brain activity. Subjects were stimulated with tACS at individual alpha frequency during eyes open and eyes closed resting states. Electrodes were placed at Cz and Oz, which should affect parieto-occipital areas most strongly. Source space magnetoencephalography (MEG) data were used to estimate phase coherence between tACS and brain activity. Phase coherence was significantly increased in areas in the occipital pole in eyes open resting state only. The lag between tACS and brain responses showed considerable inter-individual variability. In conclusion, tACS at individual alpha frequency entrains brain activity in visual cortices. Interestingly, this effect is state dependent and is clearly observed with eyes open but only to a lesser extent with eyes closed.

3.2 **Introduction**

Transcranial alternating current stimulation (tACS) is a revived and strongly used tool to investigate the causal role of brain oscillations on cognition and behavior. Many recent reviews have elucidated the importance of this tool in cognitive neuroscience (Antal & Paulus, 2103; Herrmann et al., 2013; de Graaf & Sack, 2013)

A substantial amount of evidence points to a direct influence of tACS on brain oscillations, mostly in the form of behavioral effects showing modulations of, for instance, detection ability of weak stimuli (Neuling et al., 2012a), content in working memory (Vosskuhl et al., 2015), memory task performance (Polanía et al., 2012), or crossmodal illusions (Cecere et al., 2015). All these studies applied tACS frequencies that had been linked to the investigated cognitive processes. Thus, it has been argued that tACS can show a causal relationship to cognitive function. However, this argument relies on the assumption that input waves and brain waves will

align during tACS. Considering this strong assumption, it is noteworthy that no proof in humans exists so far (but see Fröhlich & McCormick, 2010, for *in vitro* animal data).

Possibilities of investigating brain oscillations during tACS stimulation have been provided recently (Helfrich et al., 2014b; Witkowski et al., 2015). In the previous study (*Chapter 2,* Neuling et al., 2015), we focused on the proof of principle aspect of recovering a well established alpha modulation (power increase when the eyes are closed vs. open) during tACS. Here, we use the same source space data to investigate the phase relationship of tACS signal and brain oscillations during different brain states. We stimulate subjects at their individual alpha frequency (IAF, ~10 Hz). Frequencies in the alpha range have been used as stimulation frequencies in many previous tACS studies (Zaehle et al., 2010; Neuling et al., 2014; Cecere et al., 2015; Vossen et al., 2015) because their dynamics are easily observable in most subjects in parieto-occipital areas while at rest.

In this work, we aim to investigate five major assumptions about the effects of tACS: 1) It is assumed that tACS leads to phasic entrainment, i.e. an alignment of brain activity and tACS phase (Neuling et al., 2013; Thut et al., 2011) and there is evidence for entrainment of neuronal activity from animal research (Fröhlich & McCormick, 2010; Reato et al., 2010). Here, we estimate phase coherence of tACS signal and brain signal to investigate whether and how brain activity entrains online to tACS. 2) Strongest entrainment is suggested to happen in areas showing a *preference* for the entraining frequency (Rosanova et al., 2009; Thut et al., 2011). Thus, alpha frequency stimulation should be largest in visual cortex areas, the generators for visual cortical alpha. 3) Brain state dependent efficiency of tACS has been demonstrated for aftereffects (Neuling et al., 2013), such that alpha tACS affected endogenous alpha oscillations only when the subjects' alpha activity was weak (eyes open), but not so when alpha activity was strong (eyes closed). A proof for state dependent effects *during* tACS is still missing, thus, we investigated data from eyes open and eyes closed resting states to follow up on that question. 4) Considerable between-subject variation has been found in behavioral effects of tACS (Neuling et al., 2012a; Cecere et al., 2015; Riecke et al., 2015). To uncover the true tACS effects, phases have to be aligned across subjects to reveal group effects. Considering that electrical current travels through tissue instantaneously, this is surprising; however, it is likely a result of anatomical differences. We test whether entrainment of neural oscillations in visual areas during stimulation occurs with a phase offset (tACS to brain activity) in our data. This could provide a basis to explain between-subject differences in optimal phase.

3.3 **Methods**

See *Section 2.3* of the previous chapter for details on *Subjects, Procedure, tACS parameters, MEG recording and analysis.*

3.3.1 *Phase coherence*

First, epochs were averaged separately for the resting state (eyes open vs. closed) and tACS conditions (strong tACS, weak tACS, sham) for each subject. Then we obtained complex wavelet coefficients at the stimulation frequency via continuous Morlet wavelet transform between 0.5 to 1.5 s (in steps of 0.005 s) relative to epoch onset. Wavelets had a fixed width of 7 cycles. Phase (in radians) was then calculated with the Matlab function *angle*.

We computed phase coherence (Lachaux et al., 2000) between the tACS reference signal (captured at a magnetometer under the occipital electrode, MEG2121) and all virtual sensors (889 grid points) for the tACS conditions, and between an artificial cosine (at tACS frequency) and all virtual sensors for the sham condition. Phase coherence (PC) is calculated as follows:

$$PC_m = \left| \frac{1}{N} \sum_{t=1}^{N} e^{i(\Phi_{TRt} - \Phi_{nt})} \right|$$

where N is the number of time points, Φ_{TRt} the phase of the tACS reference signal and Φ_{mt} the phase at virtual sensor m at time point t. Thus, PC is coherence of the phase differences between tACS artifact and any virtual sensor over time. The same measure has recently been shown to be able to capture entrainment by tACS in the motor cortex (Witkowski et al., 2015). To estimate the temporal lag between tACS and virtual sensors, we transformed the mean phase angle differences (tACS reference signal to virtual sensors) to millisecond values by multiplying them with 1000 and dividing them by $2\pi * f$, where f is the IAF.

3.3.2 *Statistical analysis*

To test whether there was increased PC during tACS compared to sham we compared values across the whole brain with Student's t-tests (weak tACS vs. sham; strong tACS vs. sham) in the eyes open and eyes closed conditions. We used a permutation approach using cluster statistics that controls for the multiple comparison problem (Maris et al., 2007). We ran 5000 randomizations, used the maximum sum per cluster as test statistic and set the alpha level to 5%.

To compare PC across all conditions in the visual cortex (mean over voxels along the Calcarine sulcus as selected using the Automated Anatomical Labeling atlas) we computed a repeated-measures analysis of variance (ANOVA) comprised of the factors resting state (eyes open; eyes closed) and tACS condition (sham, weak, strong). The Greenhouse-Geisser correction was applied to account for sphericity violations (Geisser & Greenhouse, 1958). We report the corrected p-values and epsilon coefficient where appropriate. Post-hoc test were corrected using the false-discovery-rate (FDR) procedure (Benjamini & Hochberg, 1995).

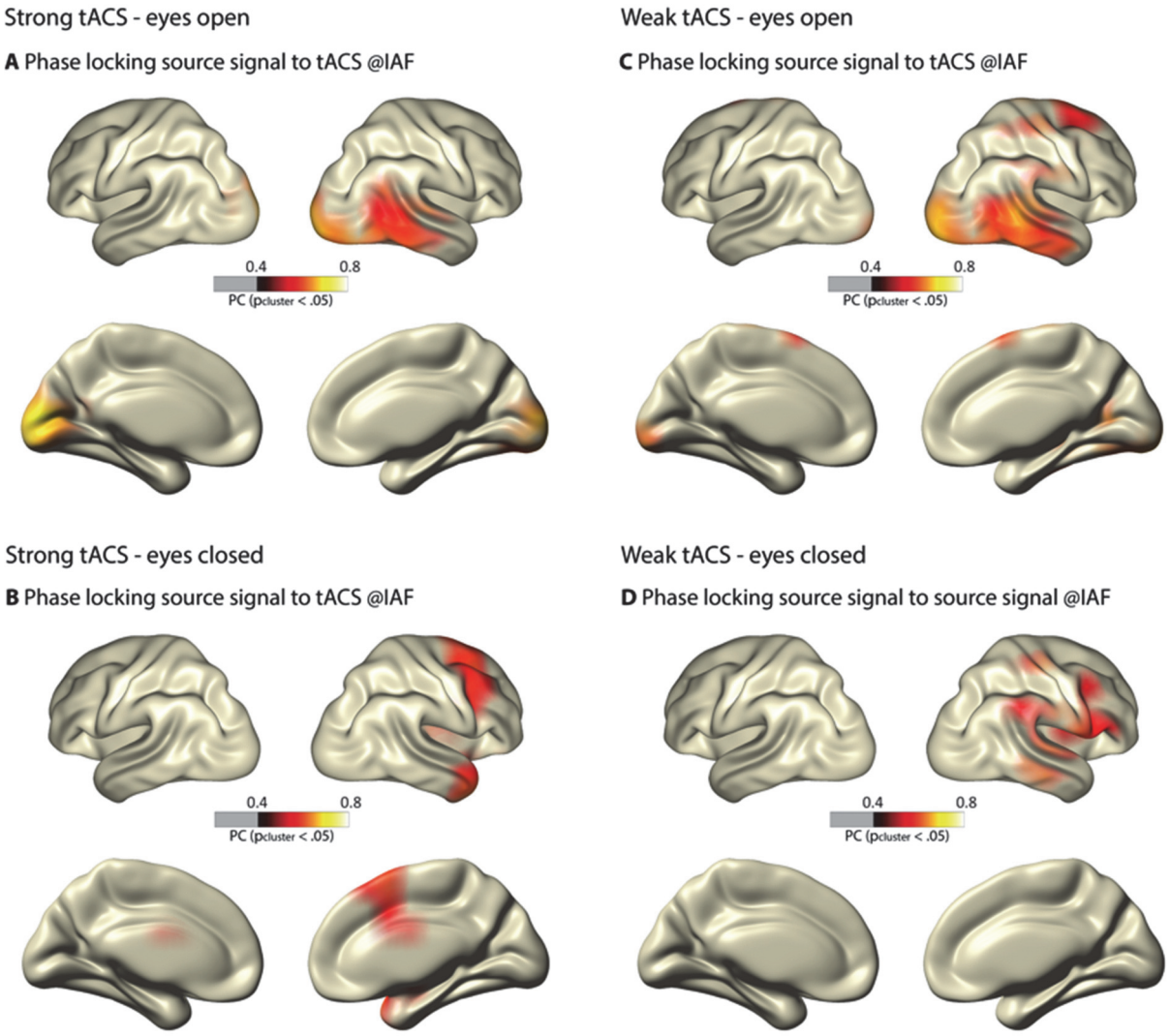


Figure 3.1 ***PC in the eyes open and closed resting state conditions for weak and strong tACS***
A and *B* show Phase Coherence (PC) during strong tACS, *C* and *D* show PC during weak tACS. *A* and *C* show the eyes open state and *B* and *D* the eyes closed state. Brain activity shows increased PC in visual cortex areas with eyes open but not with eyes closed. All cortex maps are thresholded for significant differences (cluster corrected) in PC between tACS conditions and sham. An MNI template brain was used for visualization purposes.

3.4 **Results**

With eyes open, weak and strong tACS resulted in increased PC compared to sham in the occipital pole including some right inferior temporal areas (both $p_{cluster} < 2*10^{-4}$, *Fig. 1A,C*). Coherence values were largest in visual areas.

With eyes closed in the strong tACS condition, only superior frontal regions ($p_{cluster} = 0.003$, *Fig. 1B*) showed significant PC increases. With eyes closed in the weak tACS condition, regions covering right inferior frontal, pre- and post-central areas as well as superior temporal cortex showed significant PC increases ($p_{cluster} = 0.001$, *Fig. 1D*). The ANOVA on PC amplitude in Calcarine areas (*Fig. 3.2*) showed a main effect of resting state ($F_{(1,}$

$_{16)}$ = 4.77, p = 0.044) and a main effect of tACS condition ($F_{(2, 32)}$ = 4.50, p_{GG} = 0.021, ε = 0.952). These main effects were further explained by an interaction ($F_{(2, 32)}$ = 3.56, p_{GG} = 0.042, ε = 0.961). Post hoc tests showed no differences in PC between the eyes open and eyes closed state in the sham condition ($F_{(1, 16)}$ = 0.47, p_{FDR} > 0.05) while PC was larger during eyes open compared to eyes closed in the weak tACS condition ($F_{(1, 16)}$ = 14.28, p_{FDR} < 0.01). The strong tACS condition showed a trend for the same effect (eyes open > eyes closed; $F_{(1, 16)}$ = 4.21, p_{FDR} < 0.100). Furthermore, the weak and strong tACS conditions yielded larger PC than sham in the eyes open state ($F_{(1, 16)}$ > 6.53, p_{FDR} < 0.05), however, no differences between tACS conditions (sham; weak; strong) were observed in the eyes closed resting state (all $F_{(1, 16)}$ < 3.09, p_{FDR} > 0.05).

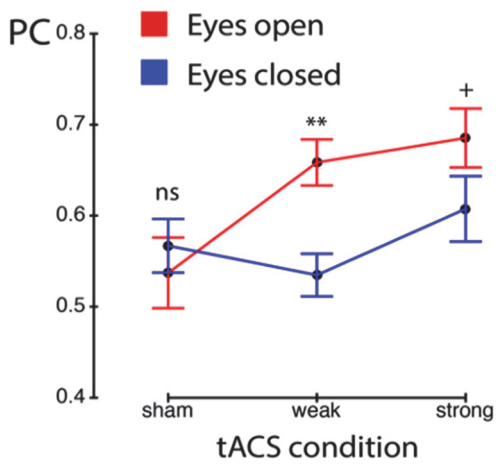


Figure 3.2 ***PC in visual cortex***
Voxels in the Calcarine cortex were selected using the Automated Anatomical Labeling atlas. Phase Coherence (PC) increases with eyes open from sham to both tACS conditions (p_{FDR} < 0.01) but no tACS condition difference was found with eyes closed (p_{FDR} > 0.05). Error bars represent the standard error of the mean. *ns*: not significant, + : p_{FDR} < 0.1, ** : p_{FDR} < 0.01.

We calculated the temporal lag in both weak and strong tACS conditions for the eyes open states, the conditions that showed significant PC in the visual cortex (*Fig. 3.3*). With strong tACS, the temporal lag in occipital areas as measured from the virtual sensor with highest PC was on average 6.0 ms, however, the variation across subjects was quite considerable (SD: 28 ms, see also supplementary *Fig. S3.1*). With weak tACS, the average temporal lag was -8.8 ms with again considerable variation (SD: 25 ms). In both tACS conditions the temporal lag was different along visual cortex areas and not one constant value.

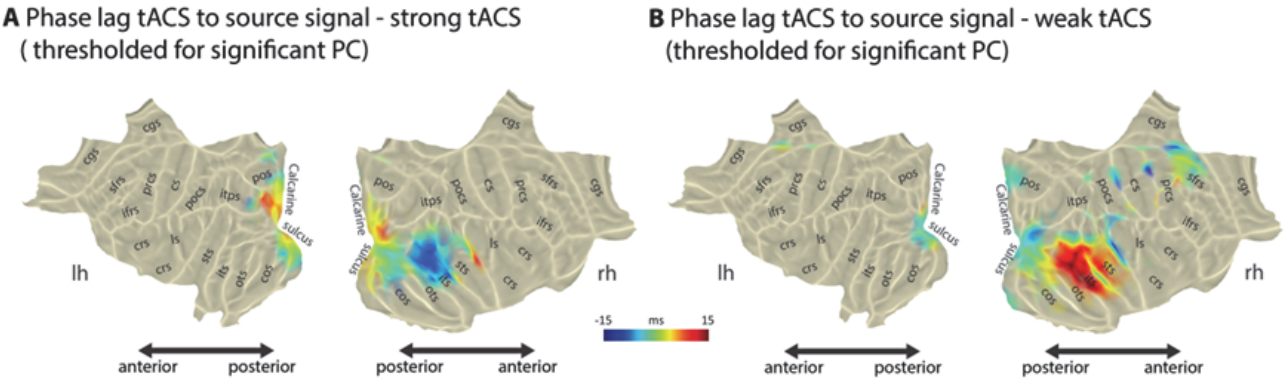


Figure 3.3 ***Phase lag of brain activity to tACS signal in the entrained regions in the occipital pole***
The phase lag shifts along the visual cortex; there seem to be no instantaneous effects in the areas that are strongly phase locked. Individual subjects' phase lag shows a substantial variation. The abbreviations for cortical sulci are based on the Mindboggle-101 dataset (Klein, 2012).

3.5 **Discussion**

The present study proves that phase locking of neural oscillations to external alpha frequency tACS emerges in the visual system. Thus, our first aim - to show entrainment of brain activity to tACS - was successful.

Our second goal was to test entrainment in regions showing a *preference* for the entraining frequency (Thut et al., 2011; Rosanova et al., 2009). Phase locking was strongest in visual cortex areas, where endogenous alpha activity can be localized. Considering that the current flow should affect broad regions along occipital and parietal areas (Neuling et al., 2012b), this regional specific entrainment is evidence for entrainment in a region preferring the alpha rhythm.

Our third goal was to show whether brain state dependent effects that have been shown for after-effects of tACS (Neuling et al., 2013; Silvanto et al., 2008) can be found online. Indeed, we see robust tACS entrainment compared to sham in visual cortex areas when subjects had their eyes open (interestingly even with weak tACS stimulation, but see below), yet, no such increase was observed in visual cortex in eyes closed resting state data. We estimated the IAF based on eyes open resting state data at the beginning of the experiment (*Chapter 2,* Neuling et al., 2015), thus, it seems natural that tACS at that frequency affects brain activity during a similar brain state. In reverse, this also means that alpha with eyes open represents a different state than eyes closed alpha, the latter less prone to perturbation by tACS. This finding might also provide an explanation under which circumstances tACS does not show effects. In a recent study (Strüber et al., 2015) no after-effects following multiple short tACS intervals (1 s) were found. The authors argue that these short intervals do not allow for neural plasticity to emerge, underlying tACS afterffects (see also Thut et al., 2011). Alternatively, they could be observing a state-dependency effect. Namely, they estimate the IAF in an eyes closed resting state

block, however, tACS is applied while the subject is performing a visual task, thus the subject is in a different state, possibly not as susceptible to tACS. Likely, this interpretation is overly simplistic, yet, it allows for creating hypotheses that need to be tested in future experiments.

Our fourth goal, investigating individual phase entrainment differences, was based on previous behavioral findings on between subject variance when investigating neural phase during tACS (Neuling et al., 2012a; Cecere et al., 2015; Riecke et al., 2015). Thus, we investigated the temporal lag between tACS and brain activity in visual cortex regions in those conditions that showed significant phase coherence in visual cortex (*Fig. 3.3*, see also supplementary *Fig. S3.1*). The entrained areas show an average temporal lag from tACS to brain oscillations of around 6 ms with strong and - 9 ms with weak tACS; however, in both cases with large variation (supplementary *Fig. S3.1*). This challenges the rather naive view that the tACS input wave can be directly translated into brain responses. Unfortunately, in the current experiment we did not record behavioral data. In future experiments, it is crucial to investigate behavioral and neural response together to see how well the temporal lag can predict the behavioral response within and across subjects. Here, we used individually adjusted tACS intensities, which could additionally explain temporal lag variation (even though in our study intensity did not correlate with temporal lag across subjects). However, further investigations are needed to explore how and if intensities can influence the tACS-brain activity lag.

Surprisingly, in data from a weak intensity tACS block (0.05 mA) also recorded during eyes open and eyes closed resting states, we found very similar patterns as with strong tACS. Specifically, we found increased entrainment in visual regions with eyes open but not with eyes closed. Such effects were not expected, considering that these intensities should not affect neural activity (Reato, 2013) - the condition was planned as control condition. However, our results clearly raise the question of how low tACS intensities can be and still have an influence on brain activity. Modelling studies with higher intensities (1 mA) suggest that low intensity transcranial electrical stimulation reaches cortical areas because the effects scale linearly (Neuling et al., 2012b). A preliminary correlation analysis on the tACS intensities across subjects (supplementary *Fig. S3.2*) shows a trend to an increase in phase coherence with increasing intensity. However, our study was not designed for this question and more modelling studies are needed as well as a thorough follow-up that varies tACS intensities within subjects to investigate this further.

In conclusion, our results provide evidence for topographically specific entrainment of brain activity to alpha frequency tACS. In particular, entrainment was observed in a state dependent manner, mainly during eyes open resting state. Interestingly, entrainment occurs with subject specific phase lags between tACS and brain activity and also different lags within the visual system. Finally, our results challenge existing ideas that low intensity tACS will not affect neural activity.

3.6 **Supplementary Material**

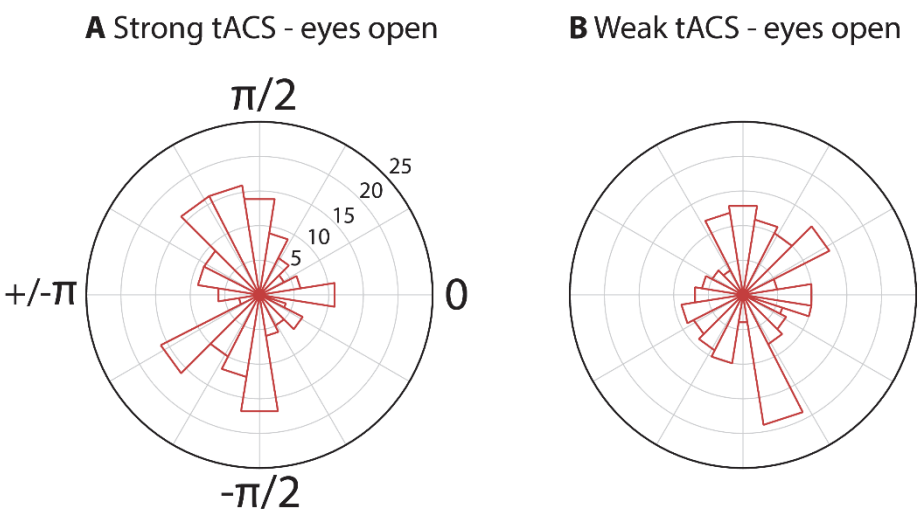


Figure S3.1 ***Histograms of phase lag tACS to brain activity in the virtual sensors in visual cortex***
Phase lag was estimated by computing the angle of the phase differences between tACS and brain activity. Considerable between-subject variation is evident with both tACS intensities.

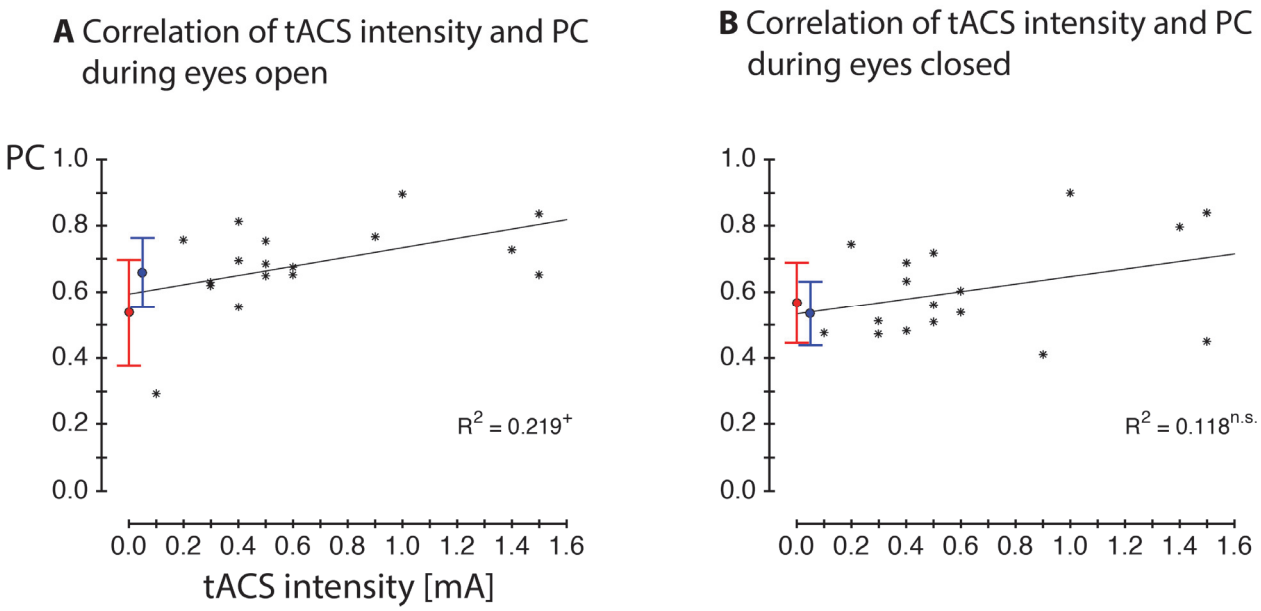


Figure S3.2 ***Correlation of tACS intensity with PC in visual cortex in the strong tACS condition***
A trend for a linear relationship is present in the eyes open resting state. Our data were not set to investigate this relationship - tACS intensity was not varied within subjects and many subjects cluster around a similar intensity of ~0.5 mA; however, these data are in line with previous data showing increasing tACS after-effects with increasing intensity and can be taken as a first indicator of the neuronal entrainment effects during tACS. The error bar at zero (red) represents mean PC and standard deviation in the sham condition. The error bar at 0.05 mA (blue) represents mean and standard deviation in the weak tACS condition.

Chapter 4. **Network-Driven State-Dependency of tACS**

The work reported here was published as preprint and summited as: Fuscà, M., Neuling, T., Ruhnau, P., & Weisz, N. (2017). Local network-level integration mediates effects of transcranial Alternating Current Stimulation. *bioRxiv, 216176.*

4.1 **Abstract**

Transcranial alternating current stimulation (tACS) has been proposed as a tool to draw causal inferences on the role of oscillatory activity in cognitive functioning and has the potential to induce long-term changes in cerebral networks. However, the mechanisms of action of tACS are not yet clear, though previous modeling works have suggested that variability may be mediated by local and network-level brain states. We used magnetoencephalography (MEG) to record brain activity from 17 healthy participants as they kept their eyes open (EO) or closed (EC) while being stimulated either with sham, weak, or strong alpha-tACS using a montage commonly assumed to target occipital areas. We reconstructed the activity of sources in all stimulation conditions by means of beamforming. The analysis of resting-state data revealed an interaction of the external stimulation with the endogenous alpha power difference between EO and EC in the posterior cingulate. This region is remote from occipital cortex, which showed strongest EC vs. EO alpha modulation, thus suggesting state-dependency long-range effects of tACS. In a follow-up analysis of this online-tACS effect, we find evidence that this dependency effect could be mediated by functional network changes: connection strength from the precuneus, a region adjusting for a measure of network integration in the two states (EC vs. EO during no-tACS), was significantly correlated with the state-dependency effect in the posterior cingulate (during tACS). No analogous correlation could be found for alpha power modulations in occipital cortex. Altogether, this is the first strong evidence to illustrate how functional network architectures can shape tACS effects.

4.2 **Introduction**

Local and long-range synchronized neural activity reflects coding and transfer of information in the brain, as studies have suggested over the years by correlating behavior with oscillatory components of electrophysiological recordings (Cohen & Kohn, 2011). In an attempt to infer more causal relationships between brain oscillations and cognition and behavior, diverse neurostimulation methods can be applied that manipulate neural activity. Among the non-invasive brain stimulation methods, transcranial alternating

current stimulation (tACS) is gaining remarkable popularity. While online effects of transcranial electrical stimulation on behavior could be investigated, brain signals during stimulation were not recoverable due to its amplitude, several orders of magnitude higher than rhythmic neuronal activity. Yet, if the stimulation artifact is consistent and non-saturating, spatial or temporal filtering can recover brain signals, even during ongoing stimulation, as some research groups have been showing for the past few years (Soekadar et al., 2013; Helfrich et al., 2014b; see however Noury et al., 2016 and Neuling et al., 2017 for a rebuttal). Neuling et al. (2015, *Chapter 2*) reconstructed brain activity from MEG recordings during simultaneously administered tACS. In this feasibility study, the authors resolved the well-known power increase in posterior alpha activity with eyes closed (Berger, 1929), showing that oscillatory effects can be recovered at the stimulation frequency. This innovation opens up the possibility to go beyond investigating *offline* aftereffects of the stimulation to understand *online* impact of tACS on brain dynamics.

State-dependent neural effects are a relevant trend established for other neurostimulation techniques (Neuling et al., 2013). This means that the cortex is more or less susceptible to the externally applied stimulation, depending on its fluctuating patterns of neural activity at local scales as well as on a network-level. State-dependency has broad implications: as the configuration of resting brain networks changes in many circumstances, most notably when damaged, treatment of neuropsychiatric disorders (Brittain et al., 2013) and non-clinical applications would benefit considerably from a clearer view of the nature of brain stimulation and its non-linear dependencies. Only a few studies have elucidated the online state-dependency of tACS. For example, a recent study by Alagapan et al. (2016) using modeling, invasive stimulation and ECoG in humans, showed that different behavioral states (such as task-engagement or resting EO / EC) profoundly impact how the same electrical stimulus perturbs ongoing activity. Non-invasively, alpha phase locking with the stimulation signal was shown to change as a function EO / EC state and current strength (Ruhnau et al., 2016b, *Chapter 3*). No study so far has pursued the issue empirically as to what extent altered network states could mediate differential effects.

Effects of tACS are seen in brain regions distant from the stimulated cortex but anatomically and functionally connected (Cabal-Calderin et al., 2016). In a framework for explaining prestimulus predispositions on conscious near-threshold perception (Ruhnau et al., 2014), we argue that network-level integration of a neural ensemble determines its propensity to impact downstream regions. In practice, network integration has been operationalized via graph theoretical metrics (Frey et al., 2016), providing support for the framework. Here, we expect that an analogous mechanism could determine how tACS could exert long-range effects. Inter-hemispheric phase synchronization has been shown to be the mechanism behind tACS interference with

functional coupling related to visual perception (Helfrich et al., 2014a). While the latter study shows tACS to affect brain connectivity, we ask how changes in functional network architectures could also shape the effect of tACS.

In the present study, we analyzed EO and EC resting-state MEG data from Neuling et al. (2015, *Chapter 2*). We expected to see online increases of power at the stimulation frequency that are dependent on brain state and tACS. In testing the notion that altered functional network could have an influence on tACS effects, we took the most consistent inter-individual modulation of network integration (in EO vs. EC, in the precuneus) in the non-stimulated brain and investigated how this relates to the state-dependent alpha power change (in posterior cingulate) during tACS. This analysis revealed a significant association, whereas an analogous analysis using power changes during EO and EC (during no tACS) to correlate with the state-dependency effect was not significant. Overall, our results yield empirical evidence that functional network modulation mediated even by an apparently simple behavioral manipulation can profoundly shape tACS effects.

4.3 **Methods**

See *Section 2.3* of *Chapter 2* for details on *Subjects, Stimuli and Procedure, tACS parameters, MEG data recording, IAF determination* and some *Offline MEG data analysis (Preprocessing* and *Source projection of raw data).*

4.3.1 *Resting-state power spectrum and PLV*

Fourier coefficients were estimated for each epoch of the resting-state data in MEG sensors and in reconstructed activity in brain sources. We used a multitaper spectral estimation (Mitra and Pesaran, 1999) with a fixed smoothing window of ±2 Hz, with a 1 Hz resolution for the frequencies between 1 and 40 Hz and 2 Hz for those between 42 and 84 Hz. The same parameters for low and high frequencies bands were chosen in order to make comparisons across the entire spectrum.

Mean power densities were determined by averaging the squared absolute value (complex magnitude) of Fourier spectra across epochs and tapers for every sensor and brain source. As a measure of entrainment, we used inter-trial phase locking value (PLV; Lachaux et al., 1999), calculated as the absolute value of the mean of complex Fourier coefficients. As trials from sensor data were aligned to the phase of the tACS, this gave us an inter-trial coherence to the stimulation at the sources. These averages were computed separately for all stimulation conditions (sham, weak and strong tACS) and for the whole recording with both EO and EC states collapsed together or taken singularly.

4.3.2 *Statistical analysis and state-dependency*

Power from different conditions was compared using nonparametric cluster-based permutation dependent-samples T-statistics (Maris & Oostenveld, 2007). Cluster randomization was repeated over 5000 permutations. We contrasted relevant conditions at frequencies of interest, alpha and its first 2 harmonics, the first sub-harmonic and 2 control frequencies in between, corresponding to 5, 10, 15, 20, 25 and 30 Hz. Only cluster-corrected p values lower than $0.008\overline{3}$ (0.05 alpha divided for the 6 frequencies comparisons) were taken into account. Dependency of tACS effects on EC and EO was assessed by computing the percent power change of strong tACS relative to no stimulation baseline within subject and condition, as follows:

$$\frac{EC_{strong\ tACS} - EC_{no\ tACS}}{EC_{no\ tACS}} \ vs \ \frac{EO_{strong\ tACS} - EO_{no\ tACS}}{EO_{no\ tACS}}$$

The same contrast was repeated for PLV and for weak stimulation. Because separate beamforming filters were used, it is not possible to draw a clear interpretation from a direct comparison between different levels of tACS on reconstructed brain sources, but this normalized contrast overcomes this limitation. For illustrative purposes, we also contrasted this way EC versus EO without stimulation and strong and no tACS conditions (both EC and EO).

4.3.3 *Graph analysis*

We calculated the source-by-source coherence from the Fourier spectra. The absolute value of the imaginary part of coherency was used as the functional connectivity metric to obtain the adjacency matrix. Imaginary coherency guards against spurious correlations due to volume conduction (Nolte et al., 2004). To find the best threshold to binarize the adjacency matrix, we started with the minimum value that ensured, for each frequency, the highest imaginary coherency without disconnected nodes in the graph. This threshold, in which every node in the graph has at least one edge, avoided underestimation of connections. A range of thresholds around this value (±0.1 in steps of 0.02) was then tested to ensure the stability of the effects across choices of threshold. The threshold used was the one that had the highest and most stable effect for nearby values.

Local connectivity for each node was assessed with the following measures: node degree, efficiency, clustering and betweenness (Rubinov & Sporns, 2010). We then applied the same nonparametric cluster-based permutation tests to these measures as we did for power (also the same frequencies, see *4.3.2 Statistical analysis and state-dependency* section above), contrasting however only EC against EO for no tACS condition. Via this analysis, we assess the individual propensity of network modulation due to this subtle behavioral change, yielding a stronger argument that potential long-range effects during tACS are mediated by network-level changes. Local efficiency was the only measure that showed a cluster at a p value lower than $0.008\overline{3}$. This index

in particular, as the inverse of the graph average path lengths of all direct connections, reflects the integration of a node in the network. The source with maximum t-value was used as a seed to get individual imaginary coherency values to the source of maximum t-value for the state-dependency contrast. These values were used in the subsequently described seed-based partial correlations.

4.3.4 *Regression and partial correlation*

We wanted to explore whether the magnitude of the state dependency effect correlated with connectivity. First, we assessed whether the different tACS intensities with which individual participants were stimulated would explain some of the variance of the effect (Ruhnau et al., 2016b, *Chapter 3*). Therefore, we extracted for each participant the percent change of the strong tACS normalized EC / EO contrast (see *4.3.2 Statistical analysis and state-dependency* section) from the source with maximum group-level t-value. We regressed the individual tACS intensity with these values. The linear model fit provided the adjusted R-squared and the F statistics.

As we ascertained the relation of stimulation intensity with state-dependency, we had to partial out the variance explained by intensity from subsequent correlations. We were interested in the area with differential network integration profile in EC and EO and its connection with the state-dependency region. We therefore took for each participant the EC / EO percent change of imaginary coherency from the source with maximal significant difference in local efficiency (see *4.3.3 Graph analysis* section above) to the state-dependency source. We then calculated the Spearman rank partial correlation coefficients between these individual percent changes of seeded imaginary coherency and state-dependency, controlling for tACS intensity. We repeated the partial correlation also with percent change of EC / EO power in the occipital cortex instead of seeded coherency, also to control for spurious correlation due to the most variance explained by tACS intensity. Correlation coefficients were t-tested against the two-sided alternative hypothesis of no partial correlation.

4.4 **Results**

4.4.1 *Power modulations during tACS*

The cluster-based statistic on 10 Hz contrasting the strong tACS against sham (calculated with separate leadfield filters) portrayed a strong increase for the stimulation condition distributed in the whole brain, maximally localized in the border between the cuneus and the precuneus (*Fig. 4.1A*). The difference was highly significant ($p_{cluster} < 0.001$), but its interpretation is unclear (see *4.5 Discussion*) and it is shown here only for illustrative purposes.

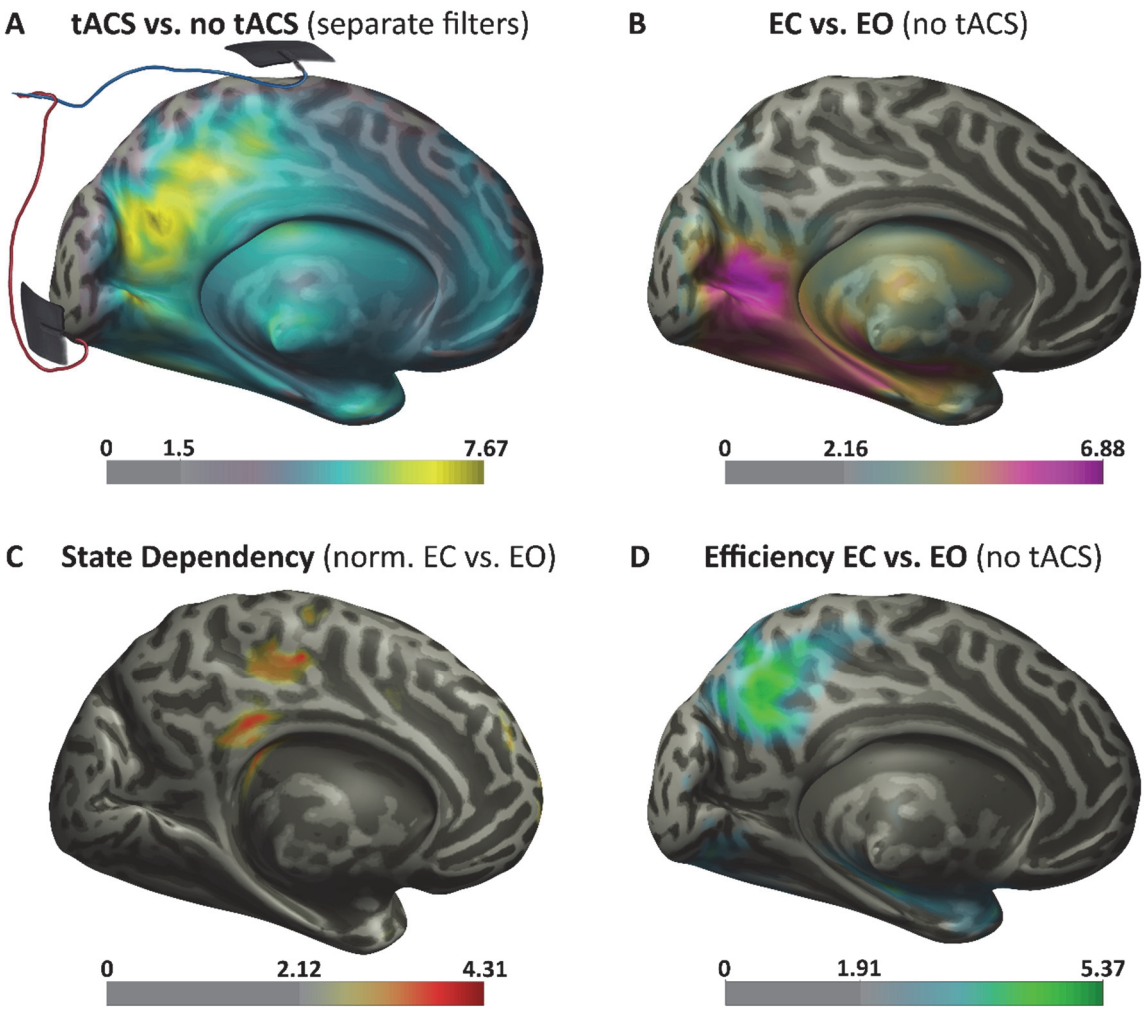


Figure 4.1 ***Posterior t-value Topographies***
Significant t-values brain maps of the principal effects at 10 Hz during EC or EO and no or strong alpha tACS. On the bottom of every MNI hemisphere, the color bars with the t-values masked for cluster-corrected $p < 0.05$ significance. Between 0 and the max is the lowest significant t-value. *A*: Medial view of the left hemisphere showing the contrast between strong and no tACS. Wired rubber patches show the location of the electrodes (attached to the scalp) with which the stimulation was delivered. The strongest effect is located in the medial parieto-occipital cortex, but an interpretation of this contrast is risky (see text). *B*: The well-known increase in alpha power in EC contrasted with EO in occipital and parietal regions. All the tACS conditions showed this difference, but here only the no stimulation contrast is shown. Alpha power differences in this region were used as predictors in the partial correlation of *Fig. 4.2B*. *C*: A stronger alpha increase for EC relative to EO during stimulation in the posterior cingulate, obtained with a normalized contrast denoting state-dependency. The max t-value source was the target of the correlations and seeded coherency of *Fig. 4.2* and supplemental *Fig. S4.3*. Unlike the other brains in this figure, here the right hemisphere instead of the left is shown. *D*: Local efficiency increase in EC relative to EO in the precuneus during no tACS. The source with the strongest contrast was the seed of imaginary coherency used in the partial correlation of *Fig. 4.2A*.

As expected, power at 10 Hz increased in contrasts between EC and EO for all tested frequencies in all stimulation conditions ($p_{cluster} < 0.002$ for no stimulation, *Fig. 4.1B*), but there was no PLV change across all conditions (supplementary *Fig. S4.2C*). The difference was maximally localized in parieto-occipital areas. Again, this well-established pattern is shown here only to illustrate the topography and its relation with the other effects.

Ongoing brain oscillatory activity interactions of stimulation and brain state was assessed with a stimulation-normalized contrast between EC and EO. No state-dependent power activity was found for this contrast for weak tACS. For strong stimulation, the cluster-based permutation test revealed a significant difference ($p_{cluster} < 0.006$) at 10 Hz and the effect was maximally expressed in the posterior cingulate (*Fig. 4.1C*). This indicates that this is the area where state-dependency at the alpha frequency was maximal, that is where the power enhancement driven by the external stimulation was modulated also by the EC brain state. The effect was only observed for power, and no significant interaction nor change in PLV was found (supplementary *Fig. S4.1B* and *Fig. S4.1C*).

At 30 Hz, the state-dependency contrast exposed a difference in power ($p_{cluster} < 0.002$), most pronounced in the right superior frontal gyrus (*Fig. S4.1D*). This online cross-frequency modulation related to EO / EC brain state was again driven by power resonance (*Fig. S4.1E*) but not by inter-trial PLV (*Fig. S4.1F*). As in the case of alpha, we cannot assert anything about the sharp increase in frontal 30 Hz power and PLV in strong tACS relative to sham stimulation, because we cannot compare tACS conditions. No other tested frequency showed state-dependent effects.

As these previous analyses established a state-dependent modulation remote to regions showing the primary power change between EC and EO (i.e. posterior cingulate vs. occipital cortex), this implied differences in neural states between these two conditions to mediate this long-range effect. This is the issue pursued in the next sections.

4.4.2 *Network-level changes between EC and EO*

To expose differences in brain network states between EC and EO, which could potentially influence this novel state-dependent effect, we used graph theoretical analysis of the resting data without tACS. The whole-brain nonparametric statistic on local efficiency uncovered a cluster in the precuneus for the 10 Hz band, which showed a significant increase in EC relative to EO ($p_{cluster} < 0.004$, *Fig. 4.1D*). Unlike the other frequency bands and the other graph measures, the effect was stable across a range of values for adjacency matrix thresholding, between 0.12 and 0.26 of absolute imaginary coherency, with a maximum effect at 0.18.

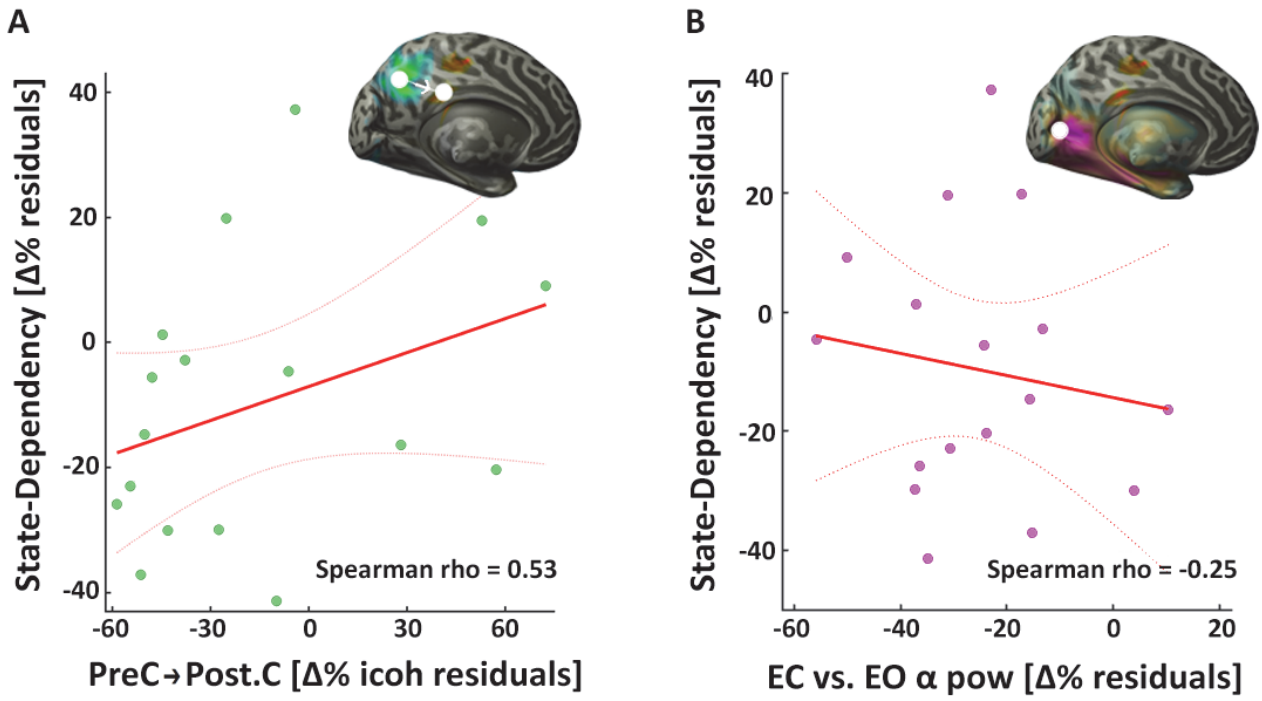


Figure 4.2 ***Partial Correlations***

Correlations and linear model fits to the individual state-dependency percentage changes. Dotted lines represent the 95% confidence intervals. *A:* Linear model fit of the adjusted values (residuals) of the partial correlation between imaginary coherency (icoh) from the source with maximum local efficiency difference, the precuneus (PreC, *Fig.4.1D*), to the state-dependency one in the posterior cingulate (Post.C, *Fig. 4.1C*, as the inset on the top right shows). The controlled variable is stimulation intensity, the independent variable in *Fig. S4.1*, here partialed-out. Again, for both the predictor icoh and the predicted state-dependency, these are individual percent change of EC vs. EO. *B*: Same as in *A*, but with alpha power increase in EC vs. EO as a predictor (extracted from the source with maximum t-value, as the inset and as *Fig. 4.1B*). This correlation is non-significant.

4.4.3 *Effects mediating state-dependency*

As participants received different tACS strength, we examined the correlation between stimulation intensity and the normalized difference in power denoting state-dependency. We found a correlation between individual intensity and the state-dependent effect (adjusted $R^2 = 0.24$, $p = 0.027$; supplementary *Fig. S4.3*).

To characterize the relationship of the underlying pattern of connectivity with the state-dependency, the source with maximum statistical value in the efficiency contrast (precuneus, *Fig. 4.1D*) was used as a seed to extract imaginary coherency to the region of the cortex with the state-dependent effect, the posterior cingulate (*Fig. 4.1C*). The partial correlation controlling for tACS intensity revealed that the connection strength between precuneus and posterior cingulate was a predictor of state-dependency (Spearman rho = 0.53, $p = 0.036$; *Fig. 4.2A*). Still controlling for intensity but using EC / EO alpha power difference as the predicting variable (extracted from the source in the occipital cortex with the strongest contrast, *Fig. 4.1B*) showed no correlation with state-dependency (Spearman rho = -0.24, $p = 0.35$; *Fig. 4.2B*).

4.5 **Discussion**

4.5.1 *State-dependent power effect of tACS*

Our analyses uncovered online interactions of tACS with brain state at the alpha frequency. Power enhancements driven by external stimulation interacted with endogenous alpha increases in the transition from EO to EC. The impact of tACS changed depending on brain state: it was more pronounced during EC in the posterior cingulate (*Fig. 4.1C*), an area between the stimulation electrodes but where current flow is not at its peak (*Fig. 4.1A*, but see below). This could be due to the dominant Eigenfrequency increase in posterior regions following closing the eyes, which overshadows the tACS influence (*Fig. 4.1B*, seen also in offline aftereffects in Helfrich et al., 2014b). That is, neural firing rate in occipital and parietal regions might still be influenced by tACS differently in the EC brain state with respect to EO, but if present, this effect is too small relative to the endogenous power modulation. On the other hand, the posterior cingulate cortex was affected differently by the stimulation, with an unclear involvement of resting networks and with consequences for task-active cognition (Leech et al., 2013). This speaks for the specificity of tACS efficacy, which is seen in certain tasks, setups and sensory modalities but not in others. The fact that it is part of the default mode circuit (with distinctive tACS network effects; Cabal-Calderin et al., 2016) and its relation with graph measures (discussed below) reinforce the idea of the singularity of this region.

When contrasting induced alpha power for different tACS conditions, there were differences distributed across the whole brain (*Fig. 4.1A*). This is difficult to interpret, as the variance was probably also driven by the different beamformer filters obtained separately from different conditions and their interaction with the reconstructed alpha power. As the filters have to suppress the artifact in the strong tACS condition, their profile is very different from the ones during no stimulation. However, one can appreciate how the cluster reflects maximum current flow of tACS located between the electrodes, as it has been modeled (Neuling et al., 2012b). Even though there is no information on tissue density in our analysis, the effect in source reconstruction shows the current diffusion where the finite-elements model predicts it.

The state-dependent effect of tACS at 30 Hz showed a frontal source distribution and again there was a higher increase in the induced power in the EC state (supplementary *Fig. S4.1D* and *E*). Interconnected areas respond, even if not directly stimulated, usually in the range of the natural frequency of their local cortico-thalamic circuit (Rosanova et al., 2009). This cluster in the right frontal pole in the second harmonic (30 Hz) follows this preferred pattern. This state-dependent interaction is reminiscent of the cross-frequency impact that tACS has on the brain, especially on multiples of the stimulation frequency (Neuling et al., 2017). It is hard to declare what drives this modulation, be it EO / EC differential frontal activity (Barry et al., 2007), resting-state network

connections from the posterior cingulate, excitability for the preferred frequency of the region (Rosanova et al., 2009), or an interaction between these factors during stimulation. The uncertain nature of this effect at 30 Hz prompted us to stop enquiring further. Yet again, harmonics and their brain state dependencies are another variable to consider when applying tACS.

4.5.2 *Effects of tACS not dependent on simple entrainment*

The interaction of tACS with EC seems to be driven only by power resonance, without a change in PLV (supplementary *Fig. S4.1B*). The posterior topography of higher alpha in EC relative to EO, seen in all tACS conditions as clearly as during no stimulation (*Fig. 4.2B*), was likewise not accompanied by phase alignment. This means that a strong continuous entrainment, in which endogenous oscillators align their phase and their frequency to the stimulation, is absent and does not contribute to power increase or online state-dependency of tACS. A trend of reduction in synchrony measured by PLV was present in the posterior cingulate but not in the parieto-occipital areas. This state-dependence of the tACS on the well-known basic alpha dynamic, adjusting when eyes are closed, is therefore unrelated with phase synchrony across trials.

This may be surprising (Herrmann et al., 2013), but there is some debate on whether entrainment is really the mechanism behind tACS effects. Many tACS studies show phasic modulation of behavior (e.g. Neuling et al., 2012a), which can be attributed to entrainment. State-dependent stimulation-driven phase alignment can be seen after trial averaging (Ruhnau et al., 2016b, *Chapter 3*). Conversely, in another online tACS study, effects on PLV (same formula used in this study) are missing, if not even dampened, at the simulation frequency (Ruhnau et al., 2016a), even though in that paradigm there were several oscillators driven at the same time.
Inter-trial phase coherence is not associated to the increase in source power, especially in this tACS setup (Neuling et al., 2013). Rather than an instantaneous synchronization of endogenous oscillators, tACS has been proposed to be altering synaptic strength, as its mechanism of action to modify brain frequencies (Vossen et al., 2014). Alternate current stimulation effects related to phase are also not seen also in recordings with implanted electrodes (Opitz et al., 2016; Lafon et al., 2017; but see Noury et al., 2017, also for a reproach on phase estimates in deep-brain recordings).

4.5.3 *Brain connectivity behind state-dependency*

Functional connectivity fluctuates continuously, but it varies mostly when the brain changes its mode of functioning. This happens during changes between different tasks or in the diverse repertoire of states like vigilance or attention. Even a simple change like closing the eyes creates profound reorganizations of brain activity. In this case, parietal cortices exhibit modifications in their oscillatory balance, especially in the low

frequency bands, closely accompanied by changes in connectivity in the same frequencies. In the alpha frequency, the most stable change seems to be the integration of the parietal cortex with the rest of the brain, which increases when eyes are closed (*Fig. 4.1D*). It is reasonable to assume that the variation in connectivity from this region to the rest of the brain is bound to influence other processes that ride on these functional connections (Ruhnau et al., 2014), such as activity modulations caused by tACS.

Reasonably, it appears that tACS influence dependent on ongoing brain state is mostly driven by the intensity of the electrical stimulation (*Fig. S4.3*). On top of that, regional variation in the system's connectivity are related to the amount of this state-dependency. Namely, the connection strength increase to this region is followed by a better integration and efficiency (*Fig. 4.2A*). Regional alpha power modulations as commonly seen during EC vs. EO, however, do not seem to mediate this state-dependent effect of tACS (*Fig. 4.2B*).

Since functional connectivity indicates electrical information flow among brain areas, it would be evocative to think that they also control the resistance of tACS diffusivity. The current flow at the neuronal scale should be the same across conditions (*Fig. 4.1A*) and changes in functional connections during different brain states reflect the distinctive responses to the impact of tACS. In other words, connectivity controls and follows patterns of inhibition and excitation in oscillatory activity, so it is likely to interact with tACS. Crucially, this is revealed in cortical regions distinct from the maximum tACS induced current.

4.5.4 *Conclusion*

As non-invasive electrical brain stimulation, particularly tACS, becomes more and more popular, caveats are bound to emerge. Although there is some convincing evidence on the reliability of tACS and its impact on behavior, its direct influence on brain processes is unknown. For one thing, the present consensus was that tACS works by entrainment and that an online alignment of endogenous oscillation would follow. Instead, in this study, we have seen how during stimulation the phase of the stimulated frequency is not impacted in this setup, and rather, tACS seems to interfere with it across trials. If a sub-neural ensemble is entrained, it is not large enough to be the dominant pattern in inter-trial PLV, but there is yet no strong support on the current situation at this level.

The main message of this study is that the online effects of tACS in the parenchyma is also defined by functional factors. In the case of tACS, oscillatory activity and connectivity are interwoven with both its ability to affect interconnected regions that are far from the focal stimulated zone and its basic impact on the cortex. As we have seen, a modulation of the functional network architecture by the simplest possible behavioral manipulation has a profound impact on tACS effects. The absence of correlation for power shows that the

effects are specifically mediated via network reorganization. This finding yields important confirmation that it is connectedness that matters in determining whether activity in a region will affect downstream areas (Ruhnau et al., 2014). As modeling and predicting effects become more complex, this should have profound implications for the use of tACS in cognitive and clinical neuroscience, i.e. one should be wary when treating patients based on results obtained in controls.

Researchers must control many variables in a neurostimulatory experiment, in which varying a single parameter can reverse its effect (Moliadze et al., 2012; Veniero et al., 2017). Alternate current stimulation is alluring: if it can manipulate physiological frequencies directly, then we can assess whether oscillatory activity actually plays a causal role in cognition and behavior. However, the state-dependency of tACS effects, as well as their nonlinearity, make these current causality assumptions naive and inadequate. Thus, it is important to take this into account, both for researchers, applying tasks with distinctive activity and connectivity profiles, and for clinicians, who often have to deal with brain systems that have altered network organizations.

4.6 **Supplementary Material**

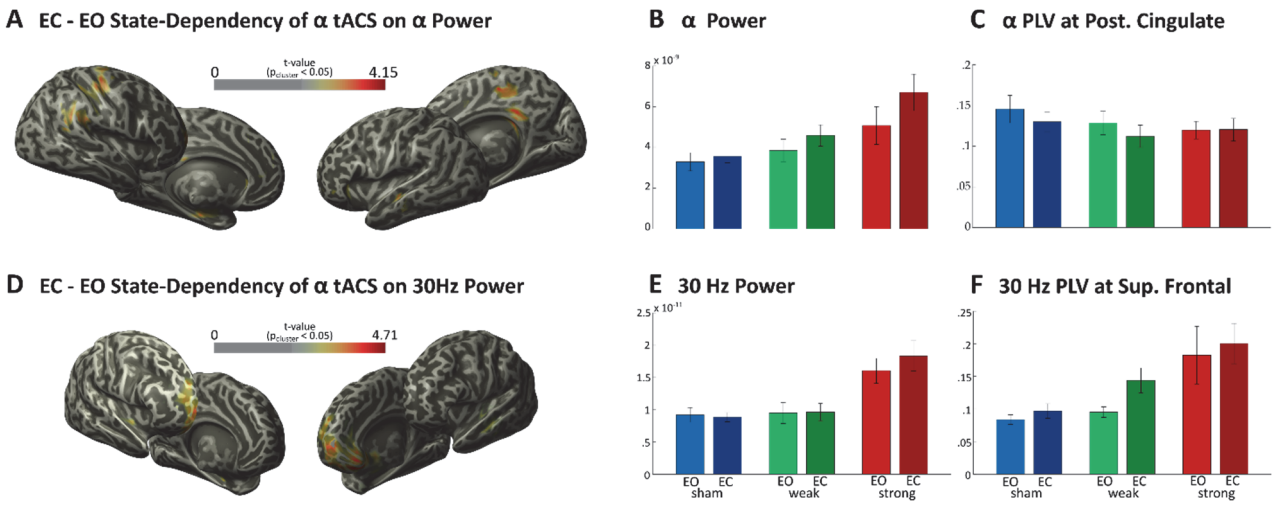


Figure S4.1 ***State-Dependent Effects***

Strong alpha tACS and EC / EO state interactions at 10 Hz and at 30 Hz. *A:* The projection on the left and right views of the left and right MNI hemispheres (left -medial- view of right hemisphere on the top right is the rotated Figure 1c) of the contrast exposing the topography of a stronger alpha increase for EC relative to EO during stimulation in the posterior cingulate. On the top, the color bar with the t-values masked for cluster-corrected $p < 0.05$ significance. *B:* Alpha Power in the virtual sensor with maximum t-value in the posterior cingulate (MNI-coordinates [10 -35 25] mm, target of the correlations and seeded coherency, Figure 2) at different stimulation and EO / EC conditions. The state-dependent effect in power capture by the normalization is discernible from the bigger EO / EC change in strong tACS. *C*: Alpha PLV at the same source. Power increase and the slight PLV decrease visible in the progressive levels of stimulation could be due to filter difference as stated before, so we cannot confidently state anything about that. *D:* Same as in *A*, but the brain is rotated to better show the EO / EC state-dependency at 30 Hz of alpha tACS in the Superior Frontal Gyrus. E. Power at 30 Hz in the Superior Frontal Gyrus source (MNI-coordinates [25 70 -5] mm) in all conditions. *F*: PLV at 30Hz at the same position. Bars indicate the condition mean and errorbars the within-subjects 95% confidence intervals.

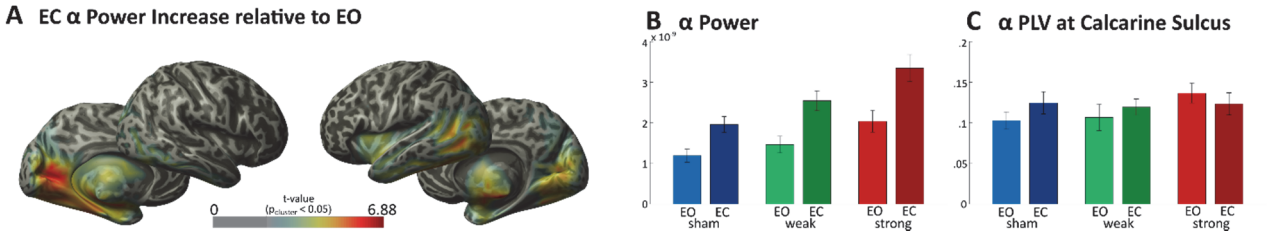


Figure S4.2 ***EC Alpha Power Increase***

Higher power in EC state relative to EO in occipital regions. *A*: Same as in Figure S4.1 above, but with the posterior topography of higher alpha power in EC relative to EO (right -medial- view of left hemisphere on the bottom left is the same as Figure 1b). *B*: Alpha Power at the Calcarine Sulcus (MNI-coordinates [-5 -95 -5] mm, max t-value, one of the seeds for coherency used in the partial correlations, Figure 2b) for the different conditions. *C*: Alpha PLV at the same location. Bars indicate the condition mean and errorbars the within-subjects 95% confidence intervals.

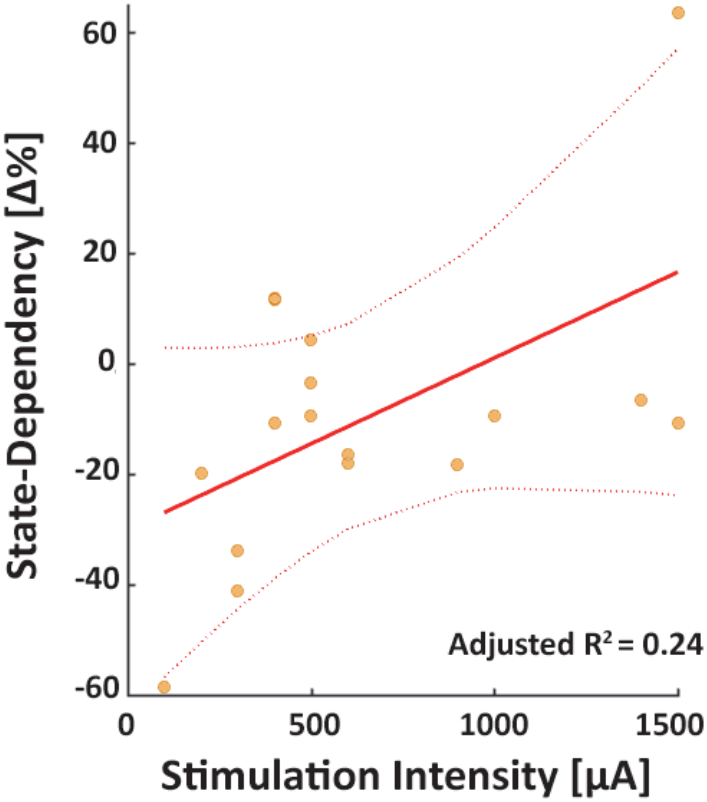


Figure S4.3 ***Regression between Intensity and State-Dependency***

Correlation between the strong tACS intensity and values for every participant of the normalized change used to detect state-dependency, in individual percent change. Dotted lines represent the 95% confidence intervals.

Chapter 5. **tACS-Driven Conscious Perception**

5.1 **Abstract**

A stimulus that is considered irrelevant by brain processing will not be consciously perceived and, even for identical information, spontaneous and momentary fluctuations in brain reactivity will determine whether it will access consciousness or not. These different states are connected to oscillatory elements of cortical activity, like the amplitude and the phase of slow neural rhythms. Here, we investigated whether we could influence and monitor this activity and human somatosensory perception directly with transcranial alternating current stimulation (tACS) and magnetoencephalography (MEG). We used a near-threshold detection paradigm paired with prestimulus tACS above the primary somatosensory cortices to modulate the predisposition to conscious perception. We found that tACS phase influences detection performance when we stimulate at alpha (around 10 Hz), but not at another control frequency. We can recover with MEG the prestimulus precursors of conscious perception during tACS, i.e. the reduction in amplitude for alpha and beta oscillatory activity. Moreover, this alpha power decrease interacts with alpha tACS, showing its dependency with a spontaneous fluctuating brain-state reflecting task-relevant sensory gating.

5.2 **Introduction**

Oscillatory neural activity is ubiquitous and has been associated with cognition and behavior (Schnitzler & Gross, 2005). Activity in the alpha band (8-12Hz) has been extensively investigated (VanRullen, 2016) and is widely accepted to reflect cortical excitability (Klimesch et al., 2007). In the context of perception, decreased levels of amplitude of the alpha rhythm have been observed prior to successfully perceived weak sensory stimuli in the visual (e.g. Ergenoglu et al., 2004), auditory (e.g. Leske et al., 2015) and somatosensory domain (e.g. Weisz et al., 2014; Frey et al., 2016). Specifically, if the alpha power level -reflecting functional inhibition- in a sensory area is relatively low, this area is assumed to be more excitable. Subsequently, an incoming stimulus is more likely to be processed and propagated to higher-order regions and thus consciously perceived.

In addition to the effect of alpha power on perception, endogenous oscillatory phase was shown to impact detection performance as well. Studies investigating the momentary phase of spontaneous alpha oscillations relative to target onset have found correlations with sensory perception. This was confirmed in a number of paradigms and sensory modalities: in the visual domain (Busch et al., 2009; Mathewson et al., 2009, 2012;

Dugué et al., 2011), auditory (Rice & Hagstrom, 1989; Strauß et al., 2015; but see Zoefel et al., 2013) and somatosensory (Palva et al., 2005; van Ede et al., 2011). Oscillatory phase and amplitude in the alpha band, both naturally fluctuating, contribute in different ways to perceptual processing. Phase reflects cortical excitability in sensorimotor regions, as a rhythmic mechanism with an inhibitory function that gates cortical activation, similarly to power but in the time scale of a few milliseconds (VanRullen, 2016). Unlike amplitude though, the role of alpha phase for information processing may be to temporally parse sensory representations (Jensen & Mazaheri, 2010), selected locally (Lakatos et al., 2008) and transmitted upstream (Fries, 2005).

The majority of such findings are correlative in nature, with contrasts of measured brain activity uncovering its relations to different perceptual conditions. Promising neurostimulatory methods, aimed to rhythmically modulate neural activity directly, include transcranial alternating current stimulation (tACS), which provides a likely avenue to tackle the study of causal links between ongoing oscillatory activity in the alpha band and perception. Manipulating electrophysiological activity while simultaneously measuring it poses a considerable challenge due to the inevitable artifact that the stimulation itself creates. Only recently, source reconstruction methods have been put forward to deal with the this problem using magnetoencephalography (MEG; Soekadar et al., 2013; *Chapter 2,* Neuling et al., 2015; Witkowski et al., 2016; but see Noury et al., 2016, 2017 and Neuling et al., 2017) or electroencephalography (EEG; Helfrich et al., 2014a).

So far, most studies have focused on the period before and after the electrical stimulation due to the strong electrical artifact. Only a few studies that employed tACS have recovered neural oscillatory activity underlying cognitive effects -such as alpha phase or power decreases prior to perception- during concurrent electrical stimulation. Concurrent MEG and tACS proof-of-principle studies settled for source reconstructing movement-related activity (Soekadar et al., 2013; Witkowski et al., 2016), well-established physiological effects such as visual evoked responses or alpha power change with open and closed eyes (Helfrich et al., 2014a; also *Chapter 2, 3, 4*), or steady-state responses (Ruhnau et al., 2016a).

Moreover, in all three sensory domains, perception was shown to be dependent on the electrically modulated alpha phase (visual: Brignani et al., 2013; Helfrich et al., 2014b; auditory: Neuling et al., 2012a; Riecke et al., 2015; somatosensory: Gundlach et al., 2016). Nevertheless, the combination of tACS and M-EEG is still in its infancy and studies employing these methods to investigate cognitive functions are rare, particularly those that aim to reconstruct source activity during the electrical stimulation.

In the current study, we set out to investigate whether it is possible to electrically modulate and recover ongoing oscillatory activity relevant for a cognitive function using tACS. Specifically, we addressed the question how tACS at 10 Hz interacts with ongoing prestimulus oscillatory activity in the alpha band during a somatosensory

perception task. We devised a near-threshold (NT) somatosensory detection paradigm and simultaneously applied tACS at 10 Hz. We added 2 control conditions, in which there was either no electrical stimulation or tACS at 35 Hz. We show, for the first time, that neural signatures of ongoing physiological processes that influence perception, i.e. alpha power modulations, can be uncovered with MEG even during electrical stimulation (at 10 Hz and 35 Hz). Moreover, the tACS-induced 10 Hz power increase was found to be state-dependent with relatively bigger power induction prior to undetected stimuli as compared to detected stimuli. Furthermore, we show that there is a prestimulus sinusoidal modulation of task performance during 10 Hz but not 35 Hz tACS, indicating that alpha tACS can be employed to alter behavior. Overall, the current study provides several important key findings. First, oscillatory activity relevant for cognition can be analysed even during electrical stimulation. Secondly, tACS interacts with ongoing processes and finally, yet importantly, somatosensory perception is influenced by the tACS-phase at 10 Hz during the prestimulus period.

5.3 **Material and Methods**

5.3.1 *Participants*

27 participants (9 females; mean age: 28 years, SD: 3 years) took part in the experiment after having given written informed consent. Overall, 4 participants had to be excluded from data analysis due to poor task performance. Either no appropriate perceptual threshold for stimulation could be identified prior to the experiment (N=3) or false alarm rates in the sham condition were too high (>25%; N=1). Thus, overall, data of 23 participants (6 females; mean age: 28 years, SD: 3 years) were analysed and are reported in this study. All participants had normal or corrected-to-normal vision. The ethical committee of the University of Trento approved the experimental protocol.

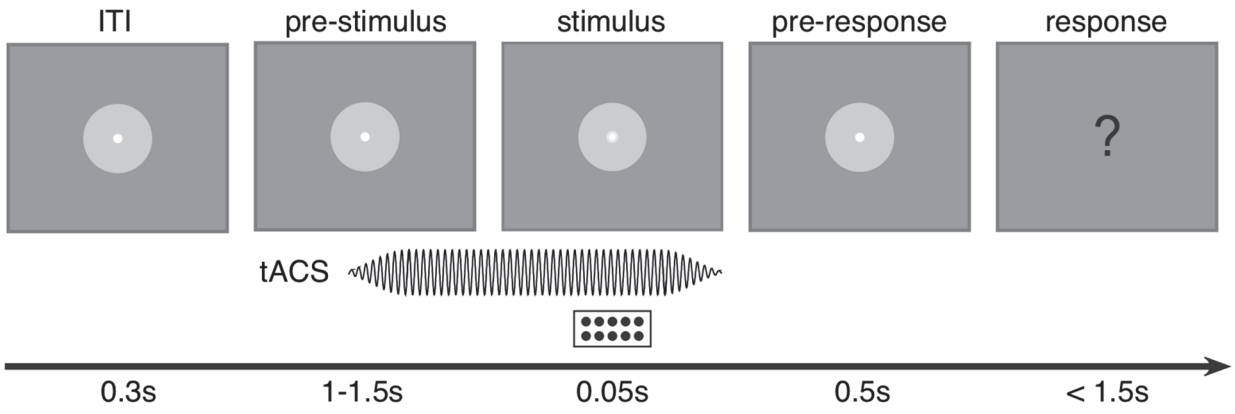


Figure 5.1 ***Tactile NT - tACS design***
Participants performed a near-threshold (NT) tactile detection task, while in some trials an alternate current stimulation was applied in the second leading to the stimulus on the left index finger. They were asked to fixate the center of the circle and, when the question mark appeared, to press a button to indicate whether they sensed the stimulus or not.

5.3.2 *Task and design*

To investigate the effects of electrical stimulation during a somatosensory perception, a well-established NT somatosensory perception paradigm was used (*Fig. 5.1*). In each trial, a tactile stimulus was delivered to the tip of the left index finger, using one finger module of a piezo-electric stimulator (Quaerosys, Schotten, Germany) with 2x5 rods which can be simultaneously raised to maximally 1mm. The module was attached to the finger with Velcro tape, and the participant's left hand was cushioned to prevent any unintended pressure on the module. To minimise eye movements, participants were asked to fixate a white dot in a larger dark grey circle cross on a light grey background throughout the whole experiment. To ensure that participants did not hear any auditory cues caused by the piezo-electric stimulator during somatosensory stimulation, binaural white noise was presented using a STIM2 system (Tip-300, Nicolet, Madison, WI, USA) and MEG-compatible tubal in-ear headphones.

Prior to the main experiment, participants performed a short training session in the MEG room, in which their individual perceptual threshold was determined using a 1-up / 1-down staircase procedure. Two randomly interleaved staircases (one going up and one downward) were used with fixed increasing and decreasing step sizes. After a short (20 trials) control and training run, the participants could continue on to the main experiment consisting of the NT somatosensory detection task. They were told that, in each trial, a weak somatosensory stimulus could be presented on the tip of their left index finger and that after each trial they would be prompted with an on-screen question to indicate whether they had felt a stimulus or not (maximal response time: 2 s). Responses were given by using MEG-compatible response boxes (VPixx technologies, Canada) with the right index and middle fingers (for yes/no, randomly interleaved between participants).

Overall, there were five to nine runs (mean: 7.7, SD: 1.2) with 100 trials each, between which participants could take breaks. Each trial started with a fixed inter-trial interval (0.3 s) and a variable prestimulus period (1-1.5 s, normally distributed) followed by a somatosensory stimulus of 50 ms, each presented either at NT intensity (experimental trials; 80 per block), clearly below threshold intensity (sham trials, 10 per block) or clearly above threshold intensity (catch trials, 10 per block). In a third of all experimental trials, no tACS was applied, whereas in the other two thirds participants were stimulated with a 1 s tACS burst either at 10 Hz or 35 Hz. The onset of the tACS was uniformly distributed between 0.85-0.75 s prior to stimulus onset such that the occurrence of the somatosensory stimulus was not associated with one specific tACS phase. After stimulus presentation, the response period was prompted by an on-screen question mark 0.5 s after the somatosensory stimulus and lasted for maximally 1.5 s. Thus, each block lasted for approximately 5 min.

5.3.3 *tACS Parameters*

Participants were stimulated with tACS before stimulus arrival while they performed the simple NT detection task. Electrical stimulation was applied with a battery-operated stimulator system (DC-Stimulator Plus, NeuroConn GmbH, Ilmenau, Germany), left outside the shielded room. Two rubber electrodes (NeuroConn GmbH) of 7 by 5 cm applied on the scalp with conductive paste (Ten20, D.O. Weaver, Aurora, CO, USA) delivered an alternating, sinusoidal current. Electrodes were placed bilaterally at C3 and C4 of the international 10-20 system, to focus the stimulation in the somatosensory cortices. The electrode cables were placed on the right of the participant's head and were connected to the stimulator via the magnetically-safe module. In order to keep participants naive to which trials the stimulation was applied, tACS intensity was kept below the individual sensation and phosphene thresholds. Each participant was tested prior to the experiment to obtain the individual stimulation threshold. For this procedure, participants were first stimulated with an intensity of 900 μA (peak- to-peak) at 10 Hz for 3 s to make them familiar with the sensation. After instructing subjects to report when the stimulation was felt, it was then repeated covertly with varying intensity, starting at 400 μA and increased by steps of 100 μA until either the subject consistently indicated the presence of tACS (always with skin sensation, never with phosphenes) or reached 1500 μA. In the cases in which the participant felt the stimulation already at 400 μA, we started at 100 μA and increased by steps of 100 μA.

5.3.4 *MEG data acquisition and preprocessing*

Prior to the experiment, the headshape of each participant was measured using a Polhemus FASTRAK 3D digitizer, relative to five coils (two on the left and right mastoid, three coils on the front). Additionally, an anatomical 3D structural image was obtained using a 4T MRI scanner (Bruker Biospin, Ettlingen Germany) from each participant. Electromagnetic brain activity was recorded using a 102 triple-sensor (two planar gradiometers and one magnetometer) MEG system (Elekta Neuromag), sampled continuously at a rate of 1kHz. All MEG data was analysed using the Matlab-based Fieldtrip toolbox (Oostenveld et al. 2011). Epochs from 3s before and 2s after the stimulus were extracted, 1Hz highpass filtered and downsampled to 512Hz. Subsequently, the data without any tACS was visually inspected to identify and remove muscular or electronic noise, channel jumps and ocular artifacts. Noisy channels that were identified based on the data without tACS were also excluded from the tACS data. After visual artifact rejection, data from all three tACS conditions was appended and aligned to the tACS onset resulting in two data files per subject (tACS-aligned and stimulus-aligned). Moreover, trial numbers for detected and undetected stimuli were equalized by random sampling for each tACS condition. These trials were then used for all subsequent analyses.

5.3.5 *Behavioral analyses*

For each participant, stimulus detection rate and false alarm rate, respectively, was computed for all three tACS conditions, the catch trials and the sham trials. Moreover, behavioral task performance was analysed relative to the phase of the 10 Hz and 35 Hz tACS by binning all trials based on the tACS onset with sampling windows and step sizes relative to the tACS frequency (sampling window: cycle length * 0.15; step size: cycle length * 0.05). Subsequently, detection rates were computed for the resulting 20 phase bins for both tACS conditions.

5.3.6 *Source-level analyses*

To analyse the MEG data also during concurrent tACS, all analyses were conducted on source-level using an LCMV beamformer approach (van Veen et al., 1997) with a regular tridimensional grid only including grey matter voxels. The grid was created based on an Montreal Institute of Neurology (MNI, Montreal, Quebec, Canada) template brain morphed into the brain volume of each participant. Realistically shaped, single-shell headmodels (Nolte, 2003) were created by co-registering the participants' headshapes either with their own structural MRI or, when no individual MRI was available (in 8 cases), with an MNI standard brain. For each tACS condition, a common spatial filter was computed based on the leadfields and the common covariance matrix from all trials, which was then used to estimate the spatial power distribution of all trials.

Spectral power was computed based on stimulus-centred data with a low-pass filter at 45 Hz using a 5 mm grid (only grey matter voxels). The LCMV beamformer filters were based on a covariance window from 850 ms pre- to 50 ms post-stimulus. Spectral power was estimated using a FFT on hanning-tapered sliding time windows of 4 cycles per frequency from 1600 ms pre- to 600 ms post-stimulus (in 50 ms steps) for 2.5-50 Hz (in steps of 2.5 Hz).

5.3.7 *Statistical testing*

5.3.7.1 Behavior

Task performance was analysed concerning stimulus detection in both control conditions (sham and catch trials) and all three experimental conditions (stimuli at threshold intensity without concurrent tACS, with 10 Hz tACS or with 35 Hz tACS). The detection rates in the three experimental conditions were compared to chance (50%) with a dependent samples t-test. Moreover, they were compared against one another (no tACS vs. 10 Hz tACS vs. 35 Hz tACS) with a one-way repeated-measures analysis of variance (ANOVA).

Task performance was analysed regarding tACS-phase dependent modulation. All trials were sampled into 20 phase bins relative to the tACS phase at stimulus onset and hit rates were computed for each bin. Phase

influences of tACS should result in a sinusoidal modulation of task performance. Thus, a sine-wave was fitted to the resulting hit rate distributions in both tACS conditions using the MATLAB routine *fminsearch* and for both model data, the coefficient of determination (R^2) was computed. Then, this value was tested for significance using a random permutation approach by shuffling the original task performance data and repeating the described procedure 10^3 times. This procedure resulted in a data-based distribution of the test statistic R^2 against which the R^2-value of the original data was tested. Additionally, for descriptive purposes only the best and worst phase angles for each participants were defined on single subject data and averaged.

5.3.7.2 Ongoing prestimulus oscillatory activity

Source-level spectral power was tested with a random effects analysis using the nonparametric SPM toolbox (SnPM; http://warwick.ac.uk/snpm). In a 1st-level test, a contrast between prestimulus alpha power in detected and undetected trials was computed within each subject. This was done for the 750-0 ms prestimulus time-window and for 10-12Hz (averaging over time and frequency) using an independent-samples t-test and a cluster-based permutation approach without correction for multiple comparisons. In a 2nd-level test, the resulting statistical maps from each participant were then contrasted in SnPM with a permutation approach with 1000 randomizations and a one-sample t-test on differences. The cluster-level threshold was set to 0.005 and the family-wise error correction to $p = 0.05$.

In addition, to test whether there was a difference between the negative alpha power effects in the prestimulus period, a within-subject ANOVA was computed in SnPM for the same time-frequency window as described above. This was done again with a permutation approach with 10^3 randomizations, a cluster-level threshold at 10^{-5} and a family-wise error correction at $p = 0.05$. For post-hoc testing, alpha power values from each tACS-condition were extracted based on a region of interest (ROI) around the second peak of the alpha ANOVA effect, which was situated in the primary somatosensory cortex (MNI-coordinates: [40 -5 55] mm). The region of interest was defined as a sphere around these MNI-coordinates with a 0.4 cm radius. After power value extraction, post-hoc tests were done between all conditions using a dependent-samples t-test.

5.4 **Results**

5.4.1 *Behavior*

Task performance across participants was analysed via response rate for sham and catch trials and for the NT experimental conditions (no tACS, 10 Hz tACS, 35 Hz tACS; see supplementary *Fig. S5.1*). For sham trials, the mean false alarm rate was 2.8% (SD: 2.3%) and for catch trials, the mean detection rate was 97.8% (SD: 2.6%). These results suggest that participants were compliant and successfully performed the given task. For the experimental trials, the mean detection rate was 50.8% (SD: 9.8%) without tACS, 49.9% (SD: 9.3%) with 10 Hz tACS, and 50.1% (SD: 10.6%) with 35 Hz tACS. None of these detection rates significantly differed from chance (without tACS: $T_{(22)} = 0.38$, $p = 0.71$, with 10 Hz tACS: $T_{(22)} = -0.04$, $p = 0.97$, with 35 Hz tACS: $T_{(22)} = 0.0.02$, $p = 0.97$), and there was no significant difference between them ($F_{(2,66)} = 0.005$, $p = 0.95$).

Sinusoidal tACS-phase-dependent modulation of task performance was tested by fitting a sine wave to the binned behavioral data and applying a random permutation approach. In contrast to the 35 Hz tACS condition (non-significant), this analysis resulted in a significant effect for the 10 Hz tACS condition ($p < 0.001$; see *Fig. 5.2*), indicating tACS phase-dependent behavior (for individual phase angles, see supplementary *Fig. S5.2).*

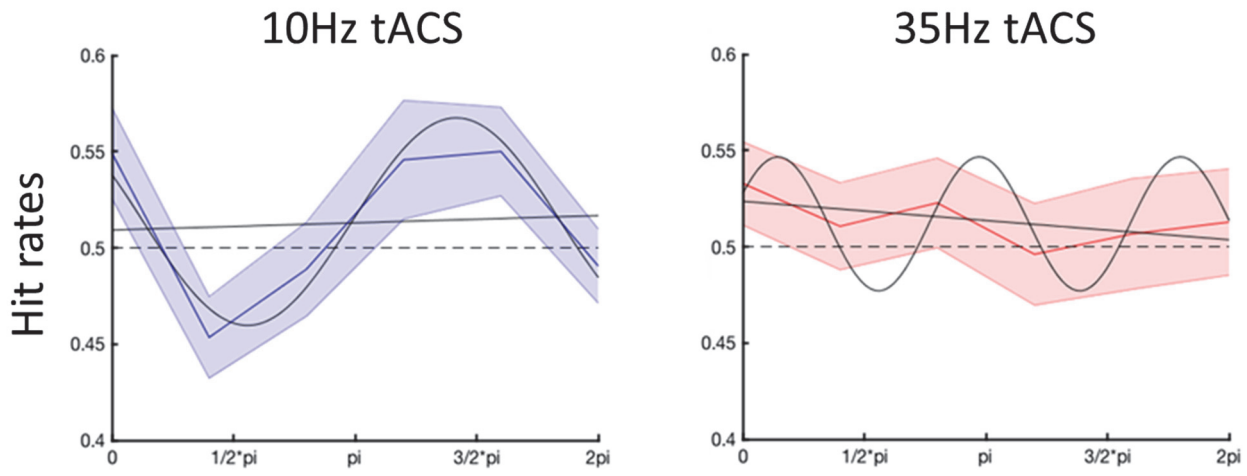


Figure 5.2 ***tACS phase modulation of performance***
NT detection rates grouped by electrical stimulation onsets show that the behavioral task performance follows a sinusoidal phase at 10 Hz during 10 Hz tACS, but no analogous effect is found for 35 Hz stimulation. The lines and the sine waves are fitted to the data.

5.4.2 *Prestimulus oscillatory power modulations*

In the current study, we aimed to investigate whether ongoing oscillatory activity could be recovered even during simultaneous tACS and whether short bursts of 10 Hz tACS modulate ongoing oscillatory activity in the alpha band. To this end, the prestimulus interval was analysed on source-level for all three NT-conditions (no tACS, 10 Hz tACS, 35 Hz tACS). The random effects analysis of the main statistical contrast of detected vs. undetected trials revealed significant alpha power decreases in the right somatosensory cortex (contralateral to stimulation) for all conditions (no tACS: p_{FWE} =0.017, 10 Hz tACS: p_{FWE} =0.011, 35 Hz tACS: p_{FWE} =0.038; *Fig. 5.3A*). A similar trend was found for beta power (see supplementary *Fig. S5.3*). The alpha power decrease in the right somatosensory cortex was strongest during the 10 Hz tACS (*Fig. 5.3B)*.

Furthermore, to test whether tACS has a modulatory effect on these single contrasts, a repeated-measure ANOVA was conducted based on the differences of detected vs. undetected stimuli. This analysis revealed a positive cluster in the right somatosensory cortex (cluster-level p_{FWE} = 0.01; maximal voxel: $F_{(1,66)}$ = 15.46, p_{FWE} = 0.005; *Fig. 5.3C*) indicating that one or more tACS-conditions significantly modulate the contrast of detected vs. undetected stimuli. Post-hoc analyses in the 2nd peak voxel of the alpha power ANOVA (in the primary somatosensory cortex; MNI-coordinates: [40 -5 55] mm) further revealed that 10 Hz tACS resulted in a relatively stronger alpha power decrease compared to the other two conditions (no tACS vs. 10 Hz tACS: $T_{(22)}$ = 2.2, p = 0.039; 10 Hz tACS vs. 35 Hz tACS: $T_{(22)}$ = -2.2, p = 0.039; *Fig. 5.3B*).

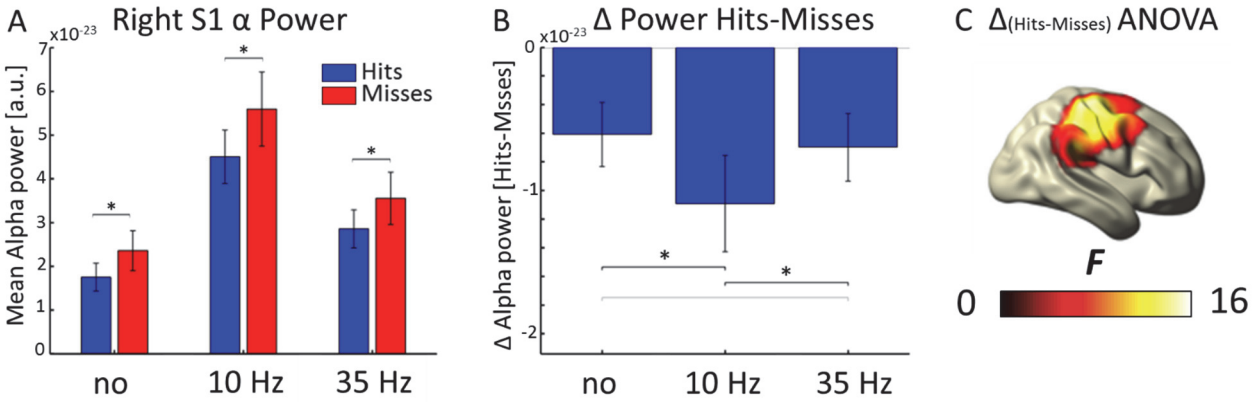


Figure 5.3 ***Alpha power difference and interaction***

Power changes in the alpha (α) band in the right (contralateral to the tactile stimulus) primary somatosensory region (S1) in the 500 ms before stimulus onset. *A.* Significant alpha power decreases in all simulation conditions (no, 10 Hz and 35 Hz tACS) in trials with detected stimuli relative to undetected ones (hits vs. misses). *B.* The hits vs. misses differences in alpha power of *A* confronted (actually post-ANOVA) revealed a significantly higher change during 10 Hz tACS than the other conditions. *C.* An ANOVA of the hits vs. misses alpha power in the 3 conditions (shown in *B*) localized the interaction again in S1. Errorbars indicate standard error of the mean. Asterisks (*) indicate a significant $p < 0.05$.

5.5 **Discussion**

The analysis of neural activity during electrical stimulation has only recently become feasible, and most studies have focused on the visual modality (Ruhnau et al., 2016a). The goal of the current study was to investigate modulations of behavior and neural activity during electrical stimulation with a particular focus on the somatosensory domain. Specifically, we set out to provide evidence that tACS-induced power modulations as well as neural activity reflecting physiological processes in a somatosensory detection task can be uncovered and adequately analysed using MEG. To this end, we combined a well-established NT detection paradigm with short intervals of electrical stimulation at different frequencies. Here, we show to our knowledge for the first time tACS-induced power increases that modulate ongoing prestimulus alpha power during a somatosensory detection task in a state-dependent manner.

5.5.1 *10 Hz tACS causes a sinusoidal modulation of task performance*

The somatosensory stimulus appeared at different moments of the cycle of the alternate stimulation. Trials' detection rates were binned according to tACS jittered onsets to evaluate its phasic influence on perceptual performance. The statistical significance of this effect, visible over all subjects only in the stimulation at 10 Hz (*Fig. 5.2*), was assessed using a nonparametric permutation test. Individual participants also exhibit a general opposition of phase bins for 10 Hz tACS (*Fig. S5.2*), speaking for comparable conduction delays of stimulus information to sensory regions between participants factoring in this effect (*Chapter 3,* Ruhnau et al., 2016b).

The alpha rhythm is involved in inhibitory mechanisms of early sensory cortices (Klimesch et al., 2007), also in somatosensory conscious perception (Palva et al., 2005), and alpha phase is relevant for the pulsed gating of information propagation (Jensen & Mazaheri, 2010; VanRullen, 2016). A phasic modulation of sensory perception of alpha tACS has already been shown before (e.g. Neuling et al., 2012a), also for NT somatosensory stimuli (Gundlach et al., 2016), but in all these previous studies the stimulation was continuous. Here, for the first time, we only target the prestimulus period, showing that alpha phase has a causal role particularly in *predisposing* to conscious perception (Weisz et al., 2014; Frey et al., 2016).

5.5.2 *tACS-induced alpha power is state-dependent*

Source reconstruction was able to recover the alpha and beta power decreases prior to the detected stimulus in the contralateral primary somatosensory region during tACS (both 10 Hz and 35 Hz) as well as during no stimulation. A decrease in prestimulus power -for detected NT targets relative to undetected ones in early sensory cortices- is a known prerequisite for conscious perception, and, in the tactile domain, there are effects in the beta band in addition to the usual alpha decrease (Palva et al., 2005; Weisz et al., 2014; Frey et al., 2016).

The sharp event-related alpha desynchronization following audio and visual stimulation was resolved during tACS with beamforming before (Neuling et al., 2015; *Chapter 2*), but this is the first time a power reduction can be seen during electrical stimulation in somatosensory regions and in the prestimulus period, i.e. in an established modulation but with a smaller effect size.

The repeated-measures ANOVA described in the previous section revealed a more extensive and stronger modulation of the prestimulus alpha power decrease during 10 Hz tACS. This modulatory effect was driven by a relatively stronger 10 Hz tACS alpha power induction prior to undetected as compared to detected somatosensory stimuli, i.e. when spontaneous alpha power was already increased. Brain state-dependency with tACS can be seen in a contrast of differences (*Chapter 4*), when the enhancement of the signal of the stimulated frequency interacts with the endogenous neural activity, which reinforces or weakens tACS effects. Here, contrary to previous investigations on tACS state-dependency, prompting eye open or closed and active tasks (Neuling et al., 2013; Alagapan et al., 2016; Ruhnau et al., 2016b, *Chapter 3* and *4*), the fluctuations in alpha power denoting the ongoing brain state are internally generated and captured by the effect they have on this task and on conscious perception.

5.5.3 *Conclusion*

Here, we set out to investigate neural oscillations during tACS using MEG. We show that tACS-induced power increases are state-dependent with relatively bigger increases if pre-tACS baseline power is already high. This state-dependency was also observed in the context of a near-threshold somatosensory detection paradigm. It is well established that undetected weak stimuli are preceded by increased alpha power. Here, electrical stimulation at 10 Hz resulted in relatively bigger alpha power increases prior to undetected somatosensory stimuli, i.e. when alpha power was already relatively increased. We can affect directly the prestimulus alpha phase, which has a consequence in the phasic gating of sensory information transfer and in the predispositions to conscious perception. Overall, the present study also provides evidence that oscillatory activity measured with MEG can be successfully analysed during tACS and that electrical stimulation interacts with ongoing physiological processes.

5.6 **Supplementary Material**

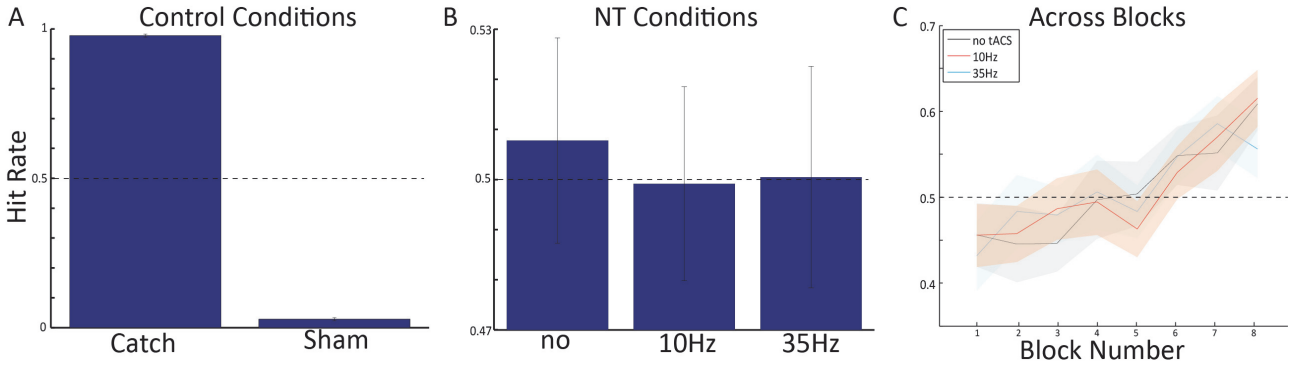


Figure S5.1 ***Behavioral performance***

Rates of correct tactile stimulus detection (hit), grouped per condition and across runs. *A.* Hit rate of the two control conditions, catch and sham, that is with a sub- or a supra- threshold somatosensory stimulation. Catch and sham trials were always without tACS. *B.* Performance for the near-threshold (NT) conditions, without prestimulus tACS (no), with tACS at 10 Hz and at 35 Hz. *C.* The small increase in tactile detection ability for NT stimuli as the experiment progressed. Errorbars and shaded areas indicate the standard error of the mean.

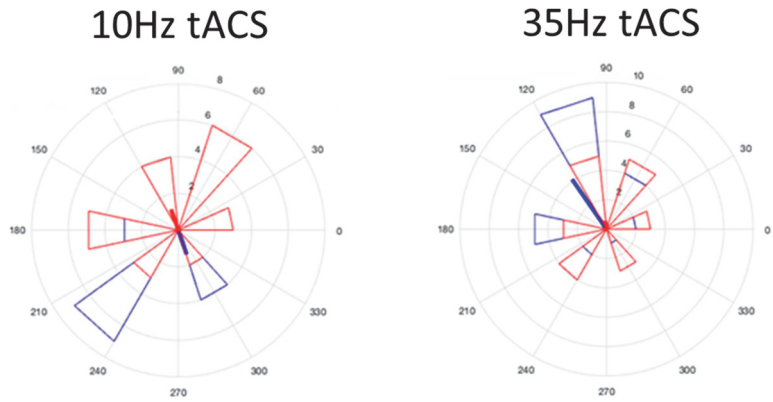


Figure S5.2 ***Individual phase angles of performance modulation***

Circular distribution of the best (blue) and worst (red) phase of subjects' fitted sine wave on detection rates in the NT task (Fig. 5.2). A significant opposite trend is present in the 10 Hz condition but not during 35 Hz tACS.

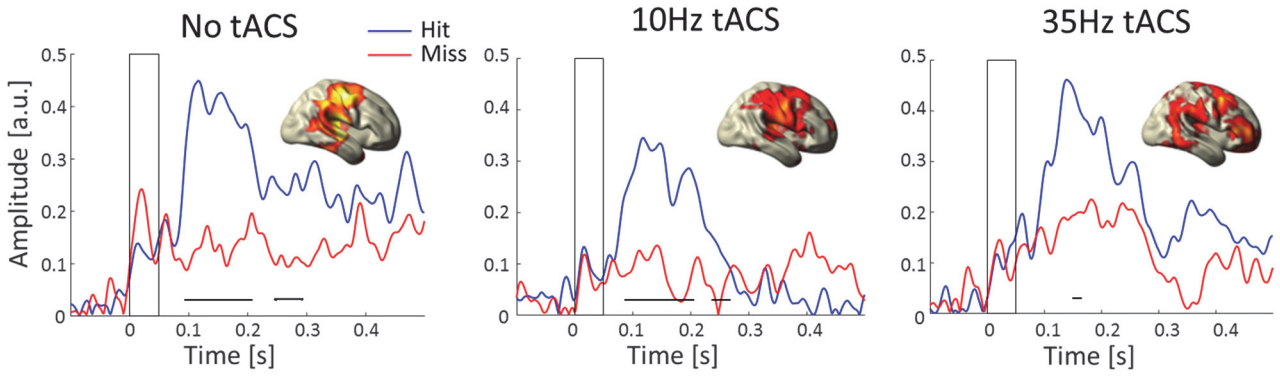


Figure S5.3 ***Event-related source activity for NT detection***

Reconstructed group-average whole-brain source activity for detected (hits, blue) and undetected (misses, red) NT tactile stimuli in the conditions without tACS, with 10 Hz and with 35 Hz stimulation. The box at time 0 until 0.05 seconds marks the tactile stimulus onset and offset. The bottom solid black lines above the time axis indicate significant time widows after the cluster-based permutation dependent-samples t-test of detected vs. undetected trials. The brain topographies represent the relative source localization in these significant time windows. For all tACS conditions, the peak of the contrast is localized around the somatosensory regions, with various degrees of SNR.

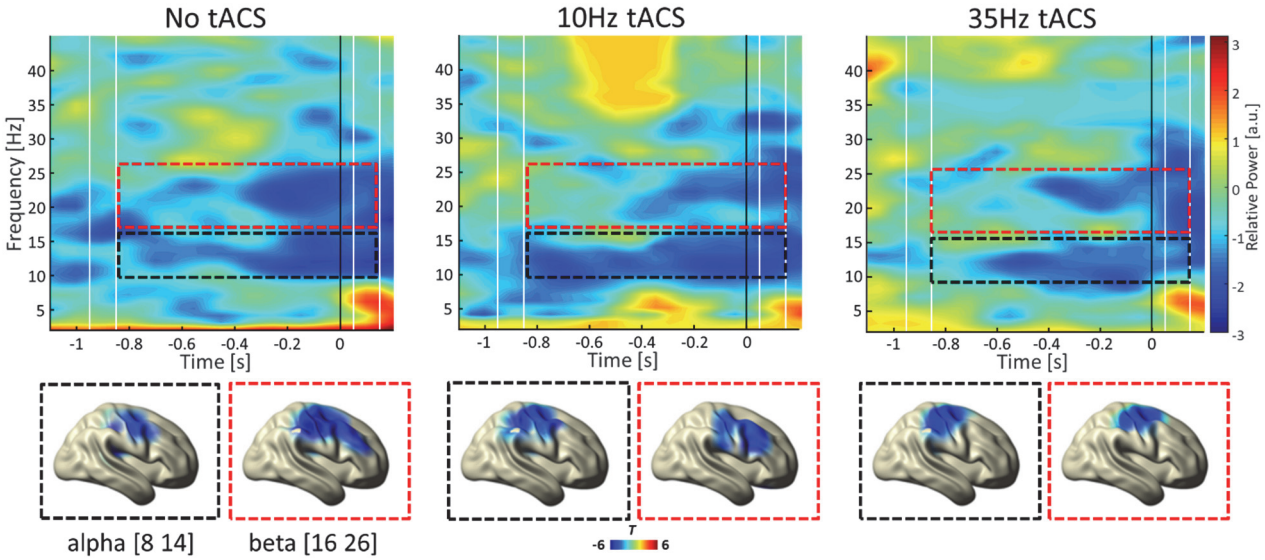


Figure S5.4 ***Source time frequency representations for NT detection***

Detected NT prestimulus power relative to undetected in the right primary somatosensory cortex in the conditions with no, 10 Hz and 35 Hz tACS. Time 0 indicates the tactile stimulus onset, the 2 couple of white lines mark the period of the tACS jittered onset and offset (minimum and maximum). The black and red dashed boxes indicate the time-frequency window for alpha (8-14 Hz) and beta (16-26 Hz), respectively, for the brain maps of the significant t-values on the bottom.

Chapter 6. **Discussion**

Information transfer in the cortex is mediated by oscillatory activity, reflecting mental states and functions. The dynamics of this complex network control, among other things, whether a stimulus will be consciously perceived or not. Fluctuating dynamical connections determine the fate of the information to be integrated in a conscious percept as a sensory stimulation arrives. Several methods aim at making sense out of the complexity that brain network represents: one technique is, for example, to perturb the system in a characteristic way that exposes intrinsic dynamics factoring in a specific function; another is a description able to capture informative features from the rich intricate levels of functional communication and network reorganization.

With brain stimulation techniques, we can investigate the causal relationship between brain rhythms and the expression of the cerebral workings on cognition and behavior. By altering neural oscillatory activity directly, as an independent variable, we can follow resulting changes in brain signal and in performance. One way to do this non-invasively is with transcranial alternating current stimulation (tACS), an electrical neurostimulatory method that uses sinusoidal current applied to the scalp. This technique is recently regaining popularity for its capacity of affecting, selectively and directly, characteristics of interest of neural oscillatory activity. Brain electrophysiological signal during stimulation was until recently difficult to recover, but it can now be extracted with spatial-filtering techniques. This casts some hope in uncovering the mechanisms of action of tACS on in-vivo brain networks, which are still unclear.

6.1 **Main Findings**

Here, we open up a line of research that sets the usage of MEG to uncover brain dynamics during tACS, aimed to bring a new perspective into the studying of conscious perception by a better understanding of how this is affected by neurostimulation. The first experiment described in *Chapter 2* is a proof of concept of this technique, in which we stimulated with tACS the alpha rhythm during standard behavioral manipulations proven to produce evoked potentials and changes in the same frequency band. In addition to evoked responses, the momentary decrease in alpha following sensory stimulation could be recovered in source space also during tACS, as well as alpha power increase when eyes are closed.

The two studies that followed took the unprecedented advantage of this method and the valuable dataset, in order to examine online effects of tACS on neural oscillations. The first of these studies in *Chapter 3* considers the average phase coherence of oscillatory brain activity to the external stimulation, showing its variability

across subjects, brain regions, stimulation intensity and conditions. The higher coherence in visual areas, the target of the stimulation, was dependent on the eyes, open or closed, as previously shown for offline aftereffects. There was also a high variability across subjects in the phase difference between stimulation and reconstructed brain activity, which could explain the individual variance in tACS behavioral results.

The next study of *Chapter 4* expanded on the finding of state-dependency and explored the actual and immediate impact of brain state on tACS-related changes. An online interaction in power mediated by neurostimulation intensity and connectivity changes, prompted by local alpha integration, was found in the parietal cortex. We framed these instantaneous effects of non-invasive rhythmic brain stimulation and their interactions in the perspective of connectivity and network theory. We showed how some of the variability of this simple online tACS state-dependency could be explained by individual differences in functional integration and communication. We also accommodated our statement of the previous chapter on entrainment and state-dependency in a broader perspective by showing no single-trial phase alignment, discussed below.

Despite confirming the purported complexity and variability behind tACS effects, we set to reveal the influence of non-invasive rhythmic brain stimulation on conscious perception. In the study in *Chapter 5,* combining tACS and MEG with an established task for near-threshold tactile detection, we uncovered the influence of stimulation on brain activity predisposing to somatosensory perception. In this paradigm, we successfully modulated oscillatory characteristics of spontaneous brain activity that have an influence on cognition and revealed them through source reconstruction. The stimulation affected the endogenous alpha decrease in the prestimulus period and consequently behavior. Ongoing alpha activity also showed an interaction with tACS, which reacted to the power that predicted the upcoming stimulus detection, again indicating its dependency on fluctuating brain states. The next step would be to examine the network reorganizations that accompany this task, tACS modulations and their interactions.

6.2 **Ubiquitous State-Dependency of tACS**

In the complex system that is the brain, an unnatural external element -like an artificial oscillatory electrical current- has a cascade of responses from the cellular to the network level (Monai et al., 2016; Alagapan et al., 2016). When applied within the safety boundaries, tACS has still negligible and temporary consequences with a limited effect size (Miranda et al., 2006; 2013; Parkin et al., 2015), visible only with repeated measures and statistics. The direction of these consequences, however, is not always consistent and their predictability is lower than ideal (Moliadze et al., 2012; Veniero et al., 2017), which raised some concerns on the reliability of electrical neurostimulation (Underwood, 2016).

The high degree of freedom (in stimulation strength, current density, electrode montage, duration, frequency, timing and phase) in tACS experiments can cause a lot of variability of the effects. To draw a parallel, TMS, with its range of coil types and their orientation, location, and stability during stimulation, together with the dependency on fluctuating cortical excitability and habituation, never suffered these controversial beginnings. Since the start, the method benefited from the visible strength of its effects and its clinical incidence, earning its validity and prevalence also in research (Sparing & Mottaghy, 2008).

However, even in an environment with all the variables controlled for, in this near-threshold conditions of efficacy due to the subtleness of tACS effects (Giordano et al., 2017), the state and the susceptibility of the cortex has a higher significance. The pre-existing baseline power of the targeted frequency has a greater impact in the outcome of the stimulation and its variability may be throwing off the linearity and the certainty of its results. This is true in resting conditions as well as in a task-active brain, as seen in this dissertation (*Chapters 3, 4, 5*) as well as elsewhere (Neuling et al., 2013; Alagapan et al., 2016). Here, we were also able to non-invasively localize where this interaction happens and evaluate its strength. Since the prevalence of state-dependency seem to be so high, taking into account the endogenous fluctuation is necessary. In this context, the ability to monitor ongoing activity during stimulation acquires new relevance. In addition to the ability to see consequences in brain activation correlated to neurostimulation, the combined approach of MEG and tACS could help controlling and predict the direction of experimental effects on oscillatory activity.

6.3 **Entrainment and its Reconstruction**

As mentioned in the introduction, there is some debate on whether entrainment, in which endogenous oscillators align their phase and their frequency to the stimulation, is really the mechanism behind online tACS effects. Many tACS studies show phasic modulation of behavior (e.g. Neuling et al., 2012a), which can be attributed to entrainment. The study in *Chapter 3* shows state-dependent stimulation-driven phase alignment following trial averaging, with a different approach described in *Chapter 4*. The latter is inspired from another online tACS study, in which effects on PLV (same procedure of *Chapter 4*) are missing, if not even dampened, at the simulation frequency (Ruhnau et al., 2016a). In that paradigm, a PLV decrease was seen at different frequencies, even though there were several oscillators driven at the same time (i.e. 11 Hz tACS PLV decrease relative to no stimulation during a 7 Hz steady-state response). These two studies differ in the measure of entrainment, one using phase coherence with the tACS signal, emphasizing overall aspects of the stimulation drive which increased (*Chapter 3*), and the other inter-trial coherence, PLV, showing a decrease (*Chapter 4*).

The interaction of tACS with EC in *Chapter 4* in fact seems to be driven only by power resonance and not by a strong continuous entrainment, as there was no change in PLV (Fig. S4.1b). The posterior topography of higher alpha in EC relative to EO, seen in all tACS conditions as clearly as during no stimulation (Fig. 2.2), was likewise not accompanied by phase alignment (Fig. S4.2b). A trend of reduction in synchrony as measured by PLV was present in the posterior cingulate but not in the parieto-occipital areas.

This may be surprising from an outdated conceptual view (Herrmann et al., 2013), but it has been noted before, as inter-trial phase coherence is not associated to the increase in source power, especially in this tACS setup (Neuling et al., 2013). The assumption of an overriding phase alignment of intrinsic oscillators during stimulation is not strongly supported (Logothetis et al., 2007; Schmidt et al., 2014). Rather than an instantaneous synchronization of endogenous oscillators, tACS has been proposed to be altering synaptic strength as its mechanism of action to modify brain frequencies (Vossen et al., 2014). Alternate current stimulation effects related to phase fail to be seen also in recordings with implanted electrodes (Opitz et al., 2016; Lafon et al., 2017).

Based on the current situation, it seems that if a sub-neural ensemble is entrained to alpha tACS (in the occipital montage) explaining previous effects on behavior and visible only following trial averaging, this is not large enough -in terms of size or strength- to be the dominant pattern when alignment to stimulation phase is calculated across trials. State-dependence of the tACS on the basic alpha dynamic, adjusting when eyes are closed, is therefore unrelated with phase synchrony across trials in *Chapter 4*. This is similar to the explanation proposed in the same chapter, stating that tACS effects are relatively weak compared to the endogenous power variability in occipito-parietal cortices, which explains the topography of the statistics that is not completely posterior.

There are some recent critiques on the neural phase of the stimulated frequency reconstructed with beamforming (Noury et al., 2017; Mäkelä et al., 2017), which undermine this secondary aspect of the findings. Even though we are cautious in stating conclusive explanations when reporting null-results about phase, we can still detect the simple power resonance following stimulation that contributes to the effects of tACS. A study expressly designed to address this issue is on its way: it uses a paradigm that has well-established entraining effects (Mathewson et al., 2009, 2012) and pairs it with tACS at the same frequency. If tACS is going to repress the effect that rhythmic stimuli have been shown to induce though entrainment on perception, it will demonstrate the ability of the current stimulation to decouple brain oscillatory activity. If instead the reconstructed neural phase does not show entrainment without an echoed reduction on behavior, that would mean that, for now, MEG with concurrent tACS is not sensible enough to detect this effect.

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